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THE RELATION OF THE LEVEL OF TRANSECTION OF THE BRAIN STEM TO THE OCCURRENCE OF DECEREBRATE RIGIDITY IN NEWBORN RABBITS¹

ALBERT M. GRIFFIN AND WILLIAM F. WINDLE

From the Anatomical Laboratory, Northwestern University Medical School

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It seems entirely probable that certain components of posture and progression must be present at the time of birth, at least in those animals upon which nature imposes the responsibilities of seeking nourishment. That the appearance of decerebrate rigidity in late prenatal and early postnatal life of the cat is one manifestation of the development of posture has recently been indicated in studies on fetal movements. At birth the kitten is capable of lifting its body up on its legs and does so to some extent in attempting to reach the mother. In accord with this, the anti-gravity reaction in the decerebrate cat and dog has been demonstrated shortly after birth (Laughton, 1924; Windle, 1929) and, in fact, it was observed in the last quarter of fetal life of the kitten (Windle and Griffin, 1931). The rabbit at birth is more helpless than the kitten and apparently its attempts to reach the mother are less purposeful. In spite of the fact that it is born in a more premature state, Laughton (1924) observed that decerebration soon after birth gave rise to extensor hypertonicity in the forelegs if the section of the brain stem were made at the proper level. His observations on newborn and young animals have been called into question because he stressed hyperextension of the limbs as the index of the presence of the phenomenon rather than actual resistance of the muscles involved in attempts to flex them passively (Langworthy, 1929).

Because of the relation of decerebration to studies on the development of the postural and progressional reactions of fetal and young animals which are being carried on in this laboratory, it was decided to extend the observations made on the dog and cat (Windle, 1929) to the newborn rabbit.

¹ Contribution no. 156.

MATERIAL AND METHODS. A series of 29 rabbits varying in age between one hour and nine days was used. Since this comprises individuals from several litters, some of which were born within a few days of each other, it was possible to compare simultaneously preparations of two or more ages. Before operation the young rabbits were placed on a warm table and their normal behavior, as well as reaction to stimulation, was observed. Particular effort was made to prevent the animals from becoming chilled during these observations and afterward they were placed in an improvised incubator where they remained until time for the operation. This was performed in a superheated room and the operating table was covered with warmed towels.

Ether anesthesia was administered to these young animals with especial care in order to avoid the shock which may ensue if it is too light and the respiratory difficulties if it is too deep. Usually, the carotid arteries were exposed and ligated and the skin approximated with metal clips. This procedure was quickly followed by reflection of the scalp and removal of the thin parietal bones. The dura mater was cleanly cut and, after exposure by shelling out the cerebral hemispheres, the brain-stem was sectioned, in different animals, at three principal levels. High "hypothalamic" sections were made in a plane passing from the cephalic half of the superior colliculi to the mamillary bodies; intermediate or "midbrain" sections were performed in one passing from the caudal half of the superior colliculi or cephalic portion of the inferior colliculi through the cephalic third of the pons; and low or "hind-brain" sections, from the caudal half of the inferior colliculi to the inferior portion of the pons. As soon as the hemorrhage had been checked by the use of small pledgets of cotton, the scalp was closed.

In the early experiments observations were made while the animals lay upon their sides. Later, in order to compare them more accurately with previous experiments, some were suspended by threads which were passed through two skin clips fastened through the skin and ligamentum nuchae and through the skin and muscles at the base of the tail.

Particular attention was paid to the presence or absence of decerebrate rigidity, the occurrence of prolonged progressive movements, and to factors of irritability in the operated specimens. Rigidity was always determined by testing the animal's ability to resist attempts to flex the hyperextended limbs. Irritability was determined by comparing its reactions to mild and strong stimulation; that is, to touching the foot pads with blunt objects or pinching with a small pair of intestinal forceps.

At the end of the experiment the animals were killed and the levels of the transections were carefully determined. The brain-stems were then removed and placed in 10 per cent formalin. Later they were imbedded in paraffin with the cut surface parallel to the end of the block, sectioned, mounted serially, and stained by the alcoholic silver nitrate method of Dav-

enport (1930). In most cases microscopic studies were made to determine the exact level of the lesion.

OBSERVATIONS. The rabbits reacted well to decerebration. Except in a few cases in which the anesthetic is thought to have been too heavy, transection was followed by a few spastic stepping and writhing movements. The animals then became quiet until anesthetic effects began to subside, at which time either progression or rigidity appeared, depending on the level at which the section had been made.

Midbrain preparations. Immediately after sectioning of the midbrain the rabbits usually executed a few writhing movements followed by alternate flexion and extension of the legs. These synergic stepping reflexes soon ceased, seldom to reappear spontaneously. In fact, prolonged progression-movements could never be obtained² and even short ones did not occur in the absence of stimulation. Decerebrate extensor rigidity, on the other hand, characterized the rabbits of this group. It usually appeared from five to fifteen minutes after operation, but sometimes it was seen almost immediately after transecting the brain-stem, depending on the depth of anesthesia. After its onset, rigidity persisted for intervals varying from thirty minutes to three and one-half hours.

Rigidity was usually first manifested by the appearance of opisthotonus, but occasionally this did not occur at all, in which case extension of the forelegs, accompanied by a certain amount of adduction, was the first indication. Although extension in the hind legs sometimes occurred almost simultaneously with that in the forelegs, definite resistance to passive flexion was invariably noticed first in the latter. There the antigravity reaction was first observed at the shoulder and elbow, after which it spread to the wrist³ and digits of those animals in which the phenomenon became most marked. In a like manner, rigidity of the hind legs first appeared at the hip joint and spread downward to the knee.

The intensity of the decerebrate rigidity is particularly worthy of note. Although a definite hyperextension was almost always evident, at times this became so marked in both fore- and hind legs that considerably more than the animal's own weight upon the limbs was required to counteract it.⁴

² In animal 9 definite synchronous running which persisted for some time was encountered and the rigidity of the legs was not at all marked. However, the plane of transection was intermediate between those which produced specimens typical of midbrain and hypothalamic sectioned ones and this may explain the fact that this animal exhibited characteristics of both.

³ Apparently tonic flexion at the wrist is part of the antigravity reflex.

⁴ In order to demonstrate that a state of true decerebrate rigidity, which could not possibly be confused with one of mild hyperextension, appeared in the animals with the midbrain sectioned, a photograph was originally submitted for publication at this place. Although it was considered desirable to omit the illustration from the paper as it appears in the Journal, it will be included in the authors' reprints as well as in the third volume of the "Publications from the Institute of Neurology" of Northwestern University Medical School.

This marked intensity was not constant in any one specimen, but diminished greatly at irregular intervals. However, if the animal were allowed to remain at rest it would recur spontaneously. Rigidity would immediately strengthen when stimulation was employed. Those animals which were suspended at the beginning of or during the observations exhibited the most constant rigidity; periods of hyperextension persisted as long as 30 minutes without any evidence of relaxation in one animal of our series. During the phases of relaxation occasional spastic stepping movements appeared. These were never prolonged but, on the contrary, lasted only a few seconds and may have been due to the irritation of the skin clips by which the animals were suspended. (They were not seen in those allowed to lie upon their sides.)

Midbrain sectioned animals were moderately irritable to ordinary stimuli. Pinching of the tail with forceps caused alternate flexion and extension of the hind legs. If the stimulus was severe, the usual response was purposeful, bilateral thrusts of the hind legs. Stepping (response to stimulation) was often prolonged for a few seconds, but was considered very short in comparison to the irritable hypothalamic rabbits which often "ran" until exhausted. The righting reflex was absent in characteristic midbrain preparations.

Histological examination of the brains of this series showed that in all but one the red nucleus had been entirely removed at operation. In one case it had been sectioned and only the caudal portion remained intact (see footnote 2).

Hypothalamic preparations. The most characteristic features of hypothalamic preparations were the occurrence of prolonged progression-movements and the persistence of the righting reflex. The animals were very irritable and responded readily to the slightest stimulation. Avoiding reactions similar to those of their unoperated litter mates occurred. Pinching the tail caused multiple bilateral thrusts of the hind legs, and pinching of the snout gave rise to a purposeful repulsion gesture of the forelegs. Irritability was so great that sudden stimulation of one ear would cause the animal to turn completely over. Between five and fifteen minutes after the operation the specimens began to exhibit spontaneous progression or running movements. These rhythmic trotting responses gradually increased in excursion and rapidity as time passed. They were present in both the fore- and hind legs despite the fact that synchronous bilateral movements of the hind legs are characteristic of progression in the adult rabbit. Early in the experiments they usually lasted from ten to thirty seconds and alternated with long periods of rest, but later the periods of activity lengthened and a corresponding decrease in the length of the rest intervals was observed until finally one continuous phase of activity ensued. When this occurred it was almost impossible to stop the animals;

they "ran" until they were exhausted. During rest intervals the limbs of the animals were usually held in the position of extension, but, if any rigidity were present, it could not be determined because the least touch on the part of the observer produced a spasm of violent activity resulting in one of the characteristic phases of prolonged running.

Histological examination was made on most of the brain-stems of this series. In no case were the red nuclei removed or cut through at operation. Other groups of cells present were the hypothalamic nuclei, nuclei proprii pedunculi, and corpora subthalamica.

Hind-brain preparations. In a manner similar to that of the previous experiments, transection through the lower part of the pons was followed by a few spastic stepping movements in the legs and writhing of the body. The animals then became quiet and assumed a crouched position. The vertebral column was arched dorsally and the legs were adducted and flexed; oftentimes the forelegs were crossed and drawn up toward the chin. The crouched position of the body was so marked that the skin of the neck and trunk was thrown into wrinkled folds which resembled a cape hanging over the shoulders and elbows. The entire body maintained a state of hyperflexion which strongly resisted passive extension; the limbs would return to the flexed position after they had been forcibly stretched. Response to stimulation was feeble and, in order to obtain any reflex movement, it was necessary to employ mutilating stimuli.

Histological examination showed that Deiter's nucleus on both sides was either removed entirely or transected at operation.

DISCUSSION. Even a casual examination of the observations recorded here shows a marked correlation between the level of the transection of the brain-stem and the occurrence of progression movements or extensor rigidity. As long as the red nuclei, hypothalamic nuclei, and associated cell groups of the midbrain and hypothalamus are left intact, decerebration of young rabbits does not produce spastic, hypertonic specimens; instead, they are extremely irritable and execute prolonged, rhythmical, running movements spontaneously. They possess, in exaggerated form, many of the characteristics of their unoperated litter mates, but seem not to have an entirely normal tonus distribution. However, if the decerebration is performed at a level just caudal to that of the red nuclei, quite a different picture is obtained; the normal behavioral reactions and the hyper-irritability then give way to the marked spasticity of the antigravity muscles which characterizes decerebrate rigidity.

The results of these experiments are in accord with those obtained by Windle (1929) on kittens and puppies; they show that the level of transection, and not the age of the animal, is the greatest factor governing the occurrence of decerebrate rigidity in young rabbits. Previous observers who have stressed the age factor have not given the proper attention to

transection levels. The plane of decerebration in Weed's (1917) kittens passed from the superior colliculi along the line of the bony tentorium; such a section would leave the red nuclei intact. Langworthy (1926) places the level of section of the rabbit brains as "just cephalad to the superior colliculi, sloping slightly forward ventrally. The thalamus, corpora striata, and hemispheres had been entirely removed; the midbrain was apparently uninjured." If these observers' animals were all transected at such levels, they correspond to the "hypothalamic" specimens of the present series, which likewise showed prolonged progression and little disturbance of normal tonus, and their efforts to obtain decerebrate rigidity in young animals failed because the sections were altogether too far rostrad. It has recently been demonstrated that adult cats will actually walk if the decerebration is performed at this level (Hinsey, Ranson and McNattin, 1930).

In the experiments of Laughton (1926) the level of transection was through the cephalic half of the superior colliculi, and a point one to two millimeters cephalad of the pons. The present observations on the occurrence of rigidity in the forelegs of young rabbits are in accord with those of this observer. The level of section in his animals was higher than that of most of those in which the best rigidity was obtained in the present series, and this may explain the fact that he did not find it present in the hind legs. Animals which for some reason did not exhibit marked foreleg rigidity invariably showed little or not any in the hind legs.

It was thought by Weed (1914) that the midbrain, and particularly the red nucleus, acted as the center for the maintenance of postural tonus, and Langworthy (1924, 1928a, 1928b, 1929) seems to have supported this hypothesis. The latter expressed the view that decerebrate rigidity does not appear in young mammals until the rubro-spinal tracts begin to myelinate. The results presented here clearly indicate that decerebration below the red nucleus does give rise to marked rigidity in young rabbits. Many observers (Thiele 1905; Cobb, Bailey and Holtz, 1917; Bazett and, Penfield, 1922; Rademaker, 1924; Pollock and Davis, 1924; Laughton, 1924, 1926; Magnus, 1925; Ranson and Hinsey, 1929; and others) have shown this to be the case in adult animals. Graham Brown (1915a) believed that the anti-gravity reaction could be observed in fetal kittens 8 to 9 cm. long and Windle and Griffin (1931) have reported its presence in several near term (considerably before the rubro-spinal tracts begin to myelinate). It is known to be present in the human infant soon after birth (Magnus and de Kleijn, 1912) and, in fact, there is some evidence that Minkowski (1923) observed it in a human fetus of 160 mm. total length. There can be little doubt that the mature red nuclei and rubro-spinal tracts are not essential for the production and maintenance of a hyper-tonicity of the anti-gravity muscles in the decerebrate condition.

Although flexor rigidity has been noted in previous studies of decerebration by Thiele (1915), Weed (1914), Brown (1915b), Bazett and Penfield (1922), Richter and Bartemeir (1926) and Davis and Pollock (1926), little study has been made on this phenomenon. Thiele (1905) thought that the center for the production of decerebrate extensor rigidity is Deiter's nucleus. He noted that when the level of transection was through fibers of the trapezoid body, or when Deiter's nucleus was destroyed extensor rigidity was absolutely abolished and sometimes flexor rigidity appeared. That a state of flexor rigidity might alternate with one of the extensor type, and that the tendency toward flexor rigidity was increased directly with the chronicity of the preparation, was observed by Bazett and Penfield (1922). Weed (1914) reported two observations of flexor rigidity. In one case an extensive hemorrhage lying beneath the whole of the cerebellar dura was found, and the other showed a three millimeter subdural clot upon the basilar plate of the occipital bone; the occurrence of flexor rigidity was thought to be associated with the hemorrhage. Davis and Pollock (1926) showed that the labyrinths exerted a strong influence upon the extensor reflex of the neck which tends to produce a fixed position of the head in extension with a consequent marked extensor rigidity in the forelegs. After destruction of the labyrinths a lasting pattern of flexor rigidity occurred in the forelegs of the animals when the head was flexed. This condition persisted until the head was again extended, at which time this rigidity was replaced by the extensor type. Histological examination showed that the level of transection in the hind brain animals of our own experiments was through the region of the eighth nerve. In view of the observations just cited it may be suggested that the phenomenon of flexor rigidity is related to destruction of vestibular centers.

SUMMARY

Decerebrate extensor rigidity occurs in newborn and young rabbits if the brain-stem is transected in a plane passing through the superior colliculi and the rostral third of the pons; the red nuclei and associated centers in the mesencephalon are separated from the functional portion of the central nervous system by such an operation.

If the plane be slanted forward to the infundibulum or mammillary bodies, the red nuclei remain intact and marked decerebrate rigidity does not occur; instead, prolonged movements of progression characterize the experiment.

It is evident that the postural reaction in young rabbits is independent of the red nucleus. Successful locomotion in these animals seems to require that this structure and possibly others in the hypothalamus remain intact.

When the level of transection is made through the hind brain in the region of the nuclei of the eighth nerve, extensor rigidity disappears and it is usually replaced by a marked flexor rigidity.

From the results of this experiment it is concluded that the occurrence of decerebrate rigidity in young rabbits is more dependent upon the level of transection of the brain-stem than upon the age of the animal.

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SOME OBSERVATIONS ON THE CIRCULATION IN EXPERIMENTAL MITRAL STENOSIS¹

JOHN H. POWERS, COBB PILCHER AND MORRIS A. BOWIE

*From the Laboratory for Surgical Research of the Harvard Medical School,
Boston, Massachusetts*

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The purpose of this report is to present observations on the cardiac output of five dogs with experimental mitral stenosis. Since the animals had been utilized previously for some work on the blood volume of normal dogs (Powers, Bowie and Howard, 1930) these studies were repeated after the development of mitral stenosis and are included. Alterations in cardiac size as demonstrated by roentgenological examination also are recorded.

The present consensus of opinion concerning heart disease in general favors the premise that the cardiac output per minute is decreased in proportion to the extent of cardiac damage. Experimental evidence to support this contention, however, is extremely fragmentary and is not sustained by the results of these investigations.

METHODS. The operative technique and the subsequent bacterial injections essential to the production of mitral stenosis have been described in detail elsewhere (Powers, 1929).

The following general statement will suffice here: the heart was exposed by subperiosteal resection of a portion of the left fifth rib. The active electrode of a portable diathermy apparatus producing a bipolar current of high frequency was approximated to the mitral ring and inferior surface of the leaflets of the valve and the current was applied several times for one to three seconds. The electrode was then withdrawn, and the wound was closed in layers with silk.

During the early post-operative period, each animal was inoculated intravenously with fresh broth cultures of streptococcus viridans.² Every dog developed accentuated cardiac sounds, a systolic murmur, and showed positive blood cultures for several weeks. As the lesions healed, organisms disappeared from the blood stream and the operation was repeated. At

¹ This study was aided by a grant from the DeLamar Mobile Research Fund.

² The strain of organisms was furnished by Dr. Hans Zinsser, to whom we are indebted for valuable advice.

the second operation some degree of stenosis of the mitral orifice and multiple scars around the ring were palpable in every case. Each animal was re-inoculated with larger quantities of streptococcus cultures than before. All developed a pronounced bacteremia presumably due to a second superimposed vegetative lesion on the mitral valve. Probably because of resistance developed in overcoming the initial infection, organisms disappeared from the blood stream relatively early as compared with the period after the first operation.

At intervals of 3 to 10 months later, the physiological observations were carried out according to the methods outlined below. All observations were made with the animal in the supine position. The legs were gently immobilized in extension by web straps attached to the sides of the table. Determinations were not carried out until the animal had been on the table approximately one-half hour.

The pulse rate was taken frequently during this resting period until a basal level was reached.

The blood pressure was obtained from the brachial artery by means of a rubber cuff attached through a closed system to a graduated mercury manometer and rubber bulb. In order to secure an accurate approximation of the cuff the inner aspect of the leg was shaved before the apparatus was applied. By this process also, extraneous sounds through the stethoscope were avoided and a more accurate reading was obtained.

With the animal under basal metabolic conditions, the consumption of oxygen was determined by the Benedict-Roth (1922) spirometer and the Blalock (1927) rubber mask.

Immediately thereafter, samples of arterial and venous blood were drawn as nearly simultaneously as practicable from the left and right ventricles or from the femoral artery and right ventricle. The skin, subcutaneous tissues and parietal pleura were infiltrated with a 1 per cent solution of novocaine before the cardiac punctures were performed. The samples of blood were deposited under oil, mixed with an anticoagulant and, as soon as possible after collection, were analyzed in duplicate in the Van Slyke-Neill (1924) constant-volume manometric apparatus for content of oxygen.

The cardiac output was determined according to the principle of Fick (1872) which states that the volume of blood passing through the lungs in cubic centimeters per minute equals:

$$\frac{\text{cc. oxygen consumed per minute}}{\text{amount of oxygen absorbed by 1 cc. of blood}}$$

The stroke output or systolic discharge was found by dividing the cardiac output per minute by the pulse rate.

The percentage of hemoglobin³ was estimated by the method of Sahli and the red blood cell counts were done with standardized pipettes and counting chambers. Venous blood was used for both procedures.

³ These determinations were also made before the animals were operated on and have been utilized in a paper entitled "Observations on the Blood of Normal Dogs with Special Reference to the Total Volume" (Powers, Bowie and Howard, 1930). The pre-operative studies are included here for the purpose of comparison.

The total volume of circulating blood was determined by the method of Keith, Rowntree and Geraghty (1915). Five cubic centimeters of blood were withdrawn from one saphenous vein and 4 cubic centimeters of a 1.5 per cent solution of Congo red were injected through the same needle. Two and one-half to three minutes later 5 cc. of blood were withdrawn from the corresponding vein of the opposite limb. Both samples were placed in round-end, glass tubes containing sodium oxalate and centrifuged at 3000 revolutions per minute for one-half hour. The hematocrit values were measured and the plasma from the second sample was read against a standard, prepared with the plasma of the first sample, in a Duboscq colorimeter. The volume of the plasma was determined therefrom by means of a formula and the total blood volume was calculated by dividing the plasma volume by the plasma hematocrit. The cell volume was obtained by subtracting the volume of the plasma from the total blood volume. These studies were carried out in the medical laboratory of the Peter Bent Brigham Hospital through the kindness of Dr. William P. Murphy.

X-ray films of the heart were exposed from a distance of one meter. The animal was allowed to lie quietly on his abdomen with the legs in extension. The plate was in contact with the anterior thoracic wall and the tube was centered over the mid portion of the dorsal spine.

Electrocardiographic tracings were made in the cardiac station of the Peter Bent Brigham Hospital through the courtesy of Dr. Samuel A. Levine.

All the above studies except the last two were repeated one or more times at varying intervals. Satisfactory determinations, checked within the limits of experimental error, thus were obtained.

In order to determine whether or not the cardiac output of a normal animal was influenced by partial resection of a rib and incision into the ventricle, the following experiment was performed. The physiological observations outlined above were carried out on dog X-20. He was then operated on in the usual manner, the electrode was introduced into the ventricle, but the current was not applied and the mitral valve was not traumatized. Two post-operative inoculations with large doses of streptococcus viridans were administered. This organism was recovered from the blood stream immediately in large numbers but at the end of two weeks the blood culture was negative. Three months later all the pre-operative studies were repeated. There was no clinical evidence of valvular disease.

RESULTS. Each of 5 dogs was subjected twice to electrocoagulation of the mitral valve followed by intravenous inoculation with cultures of streptococcus viridans. These procedures lead to the development of a chronic sclerosing lesion of the valve. Furthermore, the actual presence of such a lesion was demonstrated at the second operation when some stenosis of the orifice and multiple scars around the mitral ring were palpable in every case. In no instance was there evidence of cardiac decompensation. Except for the immediate post-operative period the animals were in good health throughout the period of study.

Observations were made on the cardiac output of these five dogs after the development of mitral stenosis. Blood studies also were carried out

TABLE 1
Dog X-3, mongrel Irish terrier, weight 14.1 kpm.

DATE	PULSE RATE PER MINUTE	BLOOD PRES- SURE	OXYGEN CON- SUMPTION	ARTERIAL OXYGEN	VENOUS OXY- GEN	OXYGEN UTILIZATION	CARDIAC OUT- PUT	HEMOGLOBIN	RED BLOOD CELLS	HEMA- TOCRIT	BLOOD VOL- UME	CELL VOLUME	PLASMA VOL- UME	CARDIAC DIAMETER BY X-RAY	REMARKS
1928			cc. per min- ute			vol. per cent	cc. per min- ute	cc. per cent	per cubic millimeter	Cells per cent Plasma per cent	cc.	cc.	cc.	cm.	
February 23	80	170/110						99.8	170,000	42.4	57.6	680	920		
March 7	80	165/95						117.8	300,000	42.4	57.6	740	1,000	6.4	Cardiac sounds normal
After two operations—each followed by intravenous inoculations with cultures of <i>Streptococcus viridans</i>															
1929															
February 16	120	180/120	122.0	22.03	18.09	3.94	3,095	25.8	7,400,000	57.0	43.0	1,695	965	730	Forceful cardiac impulse
February 21	120	190/120	109.7	22.23	18.30	3.93	2,790	23.2	110,800,000	53.4	46.6	1,645	880	765	Systolic and early diastolic murmurs
March 28	130		129.9	25.90	21.08	4.82	2,700	20.8	102,748,000	53.7	46.3	1,645	885	760	Systolic and diastolic thrills

TABLE 2
Dog X-5, mongrel spitz, weight 9.2 kgm.

DATE	PULSE RATE PER MINUTE	BLOOD PRES- SURE	OXYGEN CON- SUMPTION	ARTERIAL OXYGEN	VENOUS OXY- GEN	OXYGEN UTILIZATION	CARDIAC OUT- PUT	STROKE OUT- PUT	HEMOGLOBIN	RED BLOOD CELLS	HEMA- TOCRIT		BLOOD VOL- UME	CELL VOLUME	PLASMA VOL- UME	CARDIAC DIAMETER BY X-RAY	REMARKS
		mm. Hg	cc. per min- ute			vols. per cent	cc. per min- ute	cc. per beat	per cent	per cubic millimeter	Cells	Plasma	cc.	cc.	cc.	cm.	
1928																	
March 11	78	115/70							98.6	340,000	47.5	52.5	1,190	565	625		Cardiac sounds normal
May 31	72	120/85							96.6	460,000	47.9	52.1	1,110	530	580	6.3	Cardiac sounds normal
After two operations—each followed by intravenous inoculations with cultures of <i>Streptococcus viridans</i>																	
1929																	
April 1	84	165/115	108.1	18.88	14.04	4.84	2,235	26.6	111.7	240,000	48.7	51.3	1,245	615	630		Cardiac sounds snapping in quality
April 4	84	150/90	114.5	20.51	14.72	5.79	1,980	23.5	97.7	070,000	50.1	49.9	1,395	700	695		Soft systolic murmur
April 8	84	160/110	116.9	18.76	13.32	5.44	2,130	25.3	99.7	360,000	50.0	50.0				8.0	Diastolic thrill

and were compared with normal determinations on the same animals. The results in two typical cases are presented in detail in tables 1 and 2. Similar observations on the control dog showed no variation from the normal.

The average of three determinations of the cardiac output of each of the five animals was 2862, 2115, 4043, 2536, and 1778 cc. per minute. Expressed in cubic centimeters per kilogram per minute, these determinations become, respectively, 203, 230, 323, 149, and 104 cubic centimeters. With one exception, (323 cc. for dog X-11) these figures all fall within the range of normal values for dogs. Observations from the Vanderbilt Clinic (Blalock, 1927a, b, c) (Wilson, Harrison, and Pilcher, 1927) (Harrison and Leonard, 1926) on a series of 31 normal dogs showed the cardiac output per kilogram per minute to vary from 105 to 236 cc., the average being 163. Excluding our dog X-11, in which the cardiac output was distinctly elevated, the average output per kilogram per minute for the other four animals was 171 cc. Though this average is obtained from figures at wide variance from one another it corresponds closely to the average for normal dogs as cited above and emphasizes the fact that the cardiac output of these four animals with definitely damaged hearts was essentially within the range of normal figures. Our results are therefore different from those of Cohn and Stewart (1928) whose animals with experimental mitral insufficiency of several years' standing had outputs averaging 414 cc. per kilogram per minute. In calculating this average, one animal (no. 90) whose output per kilogram per minute was 170 cc., was omitted since he had no murmur and no increase in cardiac size. If this animal be included, the average output per kilogram per minute found by Cohn and Stewart is 373 cc., a figure still well above normal limits.

The post operative pulse rate was increased markedly in two animals and only slightly in two others. In one case there was no change.

There was no significant elevation in blood pressure except in the case of dog X-5 (table 2).

The hematocrit (the percentage of cells and plasma in whole blood) is of some interest. The percentage of cells was increased at the expense of the plasma in all but one case. It is suggested that this may be a compensatory process to increase the oxygen-carrying power of the blood in the presence of a damaged cardiac mechanism. The total cell volume, in like manner to the percentage of cells, showed a proportionately greater increase than the plasma volume, regardless of whether or not any alteration occurred in the total blood volume.

A progressive enlargement of the heart, particularly to the right, was demonstrated in all five dogs by roentgenograms. This increase, as measured by the greatest transverse diameter, varied from 7 to 27 per cent above the original measurements.

The electrocardiographic tracings showed no essential variation from the normal.

No changes whatever occurred in any of the observations in the control dog.

These five animals were subsequently subjected to a third operation on the heart involving partial resection of the stenosed mitral orifice. The studies will be reserved for a later paper.

SUMMARY

1. Observations were made on some phases of the circulation of five male dogs with experimental mitral stenosis.

2. The cardiac output of 4 of these animals ranged between 104 cc. and 230 cc. per kilogram per minute; the average was 171 cc. These figures are within the range of normal. The output of the fifth animal was distinctly elevated, being 323 cc. per kilogram per minute.

3. The basal pulse rate was increased in four instances and normal in the fifth.

4. The blood pressure was elevated in only one case.

5. An increase in the percentage of red blood cells occurred at the expense of the plasma.

6. The size of the heart as determined by the roentgen shadow showed a progressive enlargement in all five dogs.

7. No significant changes were observed in the electrocardiographic tracings.

8. Observations on the control animal showed no variations from the normal.

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THE EFFECT OF STIMULATION ON THE DEGENERATION OF A SEVERED PERIPHERAL NERVE

D. D. COOK AND R. W. GERARD

From the Department of Physiology, University of Chicago

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It is almost axiomatic in biological literature that any portion of a cell artificially separated from the nucleus will die; and the regular degeneration of the peripheral part of a severed nerve is uniformly viewed in the light of this generalization. The question may profitably be raised, however, in just what manner being attached to the cell body containing a nucleus, often some feet away, controls the viability of the peripheral nerve fiber. It is not so obvious here as in the case of the small, roundish cell why this dependence on nuclear presence should exist; or, looking at it conversely, there is here an opportunity for studying in more detail this nuclear cytoplasmic relationship. There appear *a priori* to be two possible reasons why the peripheral nerve fiber may be dependent on its connection with the cell body. It may require: a, the normal, more or less continuous passage of impulses along it to maintain its chemical activity in a normal manner—changes in their absence would be analogous to those, described as the reaction of degeneration, which occur in muscle when its innervating nerve is destroyed; or b, a direct spreading of chemical substances along the fiber. Other means than the actual movement of substances have been suggested, whereby the cell body may exert its "trophic" influence on the axone. These other possible modes of influence are not usually conceived in sufficient distinctness to permit an experimental grappling with them, and will not here be further considered.

As regards the second possibility, there exists considerable evidence that specific substances may diffuse along nerve more or less preferentially. Thus Fairbrother and Hurst (1930) have shown that polio virus injected into the cerebrum migrates down the brain stem along the pyramidal fibers, even decussating with them, and analogous results were obtained by Goodpasture (1925) with herpes virus. Le Gros Clark has similarly demonstrated the centralward diffusion of iron salts along the olfactory nerves, though not necessarily in axones; and the spread of diphtheria and tetanus toxin along peripheral nerve trunks are cases of classic interest, with evidence, again, of travel in the axones (Goodpasture, 1925).

A number of points may be considered in connection with the first possibility. When a nerve trunk is severed, the entire peripheral portion degenerates, including sensory as well as motor fibers, and the question may well be raised whether the sensory fibers do not continue to receive impulses in the normal fashion from the sense end-organs. It is conceivable, however, that the diminished motor activity will lead to fewer afferent impulses, both from exterior and proprioceptive excitation.

A more direct approach to the question is offered by the continued stimulation of the peripheral part of the severed nerve *in vivo*. Langley and Anderson (1902) and Bethe (1903) both give some evidence indicating that stimulation leads to more rapid degeneration if it does anything. The observations were, however, rather casual in the course of other work, and it seemed to us worth while more thoroughly to investigate this point. It is also worth recording, in this connection, the very general clinical experience that injured nerves are further disturbed by activity. For example, in the treatment of alcoholic neuritis or polio-myelitis, rest is a very important element, as continued activity appears to lead to greater ultimate damage.

In the present experiments, electrodes were buried along the course of the nerves, cut or uncut, and the effect of stimulation on the subsequent history after section studied. Incidentally, much interesting material was obtained in relation to degeneration time of nerve versus muscle, change in threshold, and relation between physiological and histological changes. These will be discussed in proper sequence.

METHODS. 1. *Operation.* Dogs were operated under strictly aseptic precautions. One, or usually both, sciatic nerves were exposed by incisions of the lateral lower thigh, through the fascia lata at the line of decussation of its fibers, and separation of the muscle bundles lying below. The entire sciatic was sectioned as high as possible, at the level of the neck of the femur; and when both were cut, the operation was arranged so that the actual sections were performed within a few minutes of each other. The peripheral stump was then sewed to subjacent muscle fascia. The nerve was dissected from its bed only the necessary minimum, and only at the levels of section and electrode application. Electrodes of the type to be described were placed about the nerve some centimeters (usually 2.5 to the nearest) distal to the cut and over one centimeter from each other, care being taken to disturb the nerve as little as possible and to avoid the blood vessels running through its sheath. In cases where a separate shield was placed about the electrodes, the nerve was dissected for a greater length. In some experiments the electrodes were placed still further peripherally, on either the tibial or the peroneal nerve alone, or separate ones on each. After the electrodes were placed, all bleeding was carefully stopped and the

wound closed with three rows of sutures, leaving the ends of the electrodes projecting through the skin.

2. *Electrodes.* After considerable experimentation, the following form of electrode was found simple and very satisfactory. It is easy to make, well-insulated, sterilizable and, though readily placed on the nerve, cannot come off. When properly placed, it does not disturb the blood supply nor mechanically interfere with the nerve. (When animals are allowed to walk about, a very fine enameled wire wound around the nerve is safer for mechanical reasons.) Control experiments have shown that such electrodes, even with shields, have not produced noticeable injury of nerves when left in place for two months.

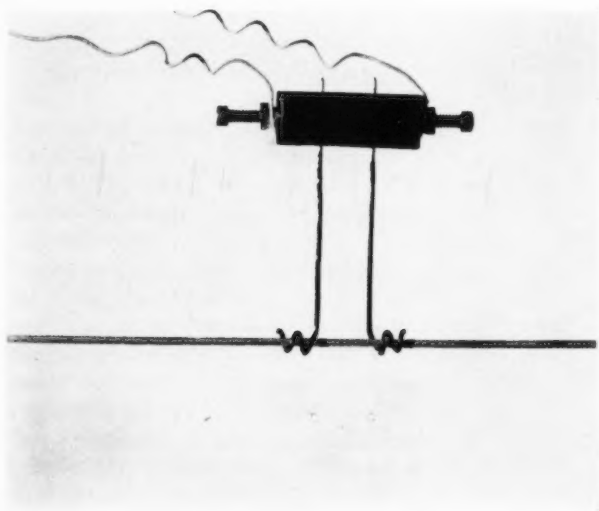


Fig. 1

Gauge 16 hard silver wire is wrapped around a rod to give two coils, 2-4 mm. in diameter. One end of the coil is sharply cut off, the other extended as a straight rod of 8 cm. (fig. 1). All of the wire but 2 cm. at the end of the straight part is next dipped in bakelite varnish and hung coil down for one hour in a drying oven kept at 80°. After cooling, the bakelite inside of the loop is burnished off to expose the silver electrode, for contact with the nerve, and the whole then baked another hour at 100-150°C. to completely harden the bakelite and to insure its sterility. The coiled end is easily maneuvered so as to cause the uncut nerve to pass through the centers of the two loops, and when so placed can only be removed by a

similar definite series of motions. The nerve thus lies in contact with some portions of the inner, uninsulated regions of the loops, all the remaining buried portion of the electrode being entirely insulated. Two such electrodes were placed on each nerve, usually 12 mm. apart, and a block, shown in figure 1, clamped on the projecting ends for contact with the stimulating apparatus and to hold the electrodes firmly in position. This

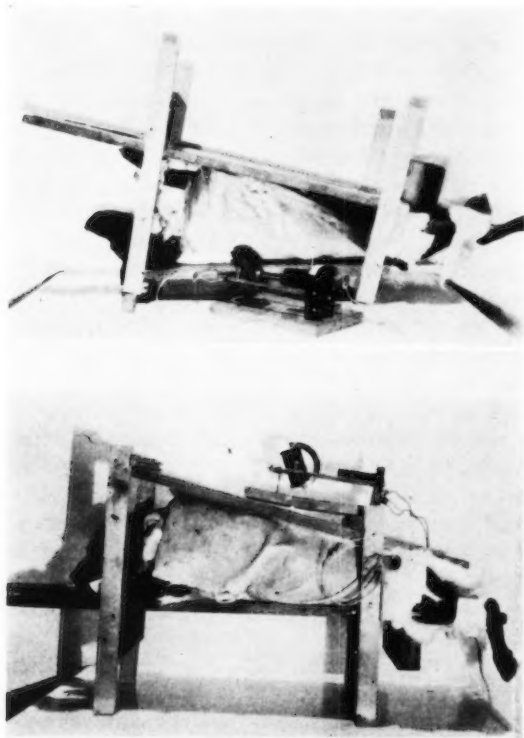


Fig. 2

block was further taped loosely to the leg by adhesive. In some cases a flexible celluloid shield four centimeters long was wrapped about the nerve and penetrated by the two electrodes. This had the advantage of entirely eliminating current-spread to adjacent muscle bundles, but the disadvantage of requiring a greater dissection of the nerve for its placing.

3. *Care of animals.* Experiments with the wires from the electrodes fastened along the dog's body and through a collar and allowing the dog

to walk around proved impracticable, as the animal eventually infected the wound. The procedure was therefore adopted of keeping the dogs mildly narcotized throughout the period of observation; 0.225 gram of sodium barbital per kilo was given by stomach tube an hour before the operation, and additional doses of 0.05 to 0.125 gram per kilo given as needed thereafter, usually about every 12 hours, to maintain the dog quiet. The barbitalized animal was kept immobilized in a dog board of the form shown in figure 2. This permitted the accurate recording of contractions of various leg muscles and facilitated the feeding and care of excretions of the animal. It further permitted regular changes in position from ventral to lateral to dorsal, to avoid the very serious complications of hypostatic edema or pneumonia. The two boards were adjusted so as to fit the pelvic region rather snugly, but allow plenty of room over the thorax. The hind legs were fixed with adhesive about cotton pads, as indicated, and the thighs supported against padded wooden blocks to prevent movement of the hips. Feces were collected in a paper bag suspended between the hind legs, urine cared for in females by catheterization. The animals were wrapped with cloth and padded with cotton to avoid decubitus and to maintain body temperature. Rectal temperature was taken regularly and did not fall below normal in any case.

The animals were given sufficient food by stomach tube to supply 40 calories per kilo per 24 hours: 10 calories of peptone and 30 of glucose. The glucose was administered every 12 hours, usually with the barbital, the peptone every 24. Five hundred to 700 cc. of warm water were also given every 12 hours. Administering large amounts of water seemed very valuable in maintaining the dogs in good condition, and also lowered the state of anesthesia produced by any particular quantity of barbital. In spite of the great nursing care given these dogs, there was considerable loss from hypostatic edema and pneumonia. This was especially true in the winter, due to changes in temperature in the room and poor initial condition of the animals. The most effective measures in combating this were large quantities of water and, particularly, the frequent change of position of the dog (and board) with respect to gravity.

Though premature deaths greatly increased the labor of this study, a number of animals sufficiently large to yield significant results was kept alive for a much longer period than that required for complete degeneration of the severed nerve. A few control experiments indicated that barbital did not significantly affect the rate of degeneration of the nerve. In no immobilized dog was infection at the region of the nerve observed, although in one or two cases infection under the skin had appeared by the time of death.

4. *Determination of irritability.* Movements of the foot were recorded on a smoked drum by means of a long aluminum wire lever attached to the

lateral toe. The foot extensor muscles could be stimulated directly by silver wires, parallel to each other, imbedded in them. The nerve was excited by means of the buried electrodes described above. For determination of threshold three methods were used. The usual Harvard induction coil was activated by 3 volts in the primary, and the secondary, set at 12 cm., was fitted with a protractor and indicator to measure exactly the coil angle. The greatest possible angle from the horizontal giving a minimal twitch was taken as the threshold value. Second, a coreless induction coil was used with the secondary directly over the primary, and a resistance in the primary circuit varied. The maximal resistance just giving a twitch was again the threshold. In this case 1.5 volts were used in the primary. The shock strength (total current) is inversely proportional to the primary resistance with the coreless coil. The relation of coil angle to stimulus current was determined by connecting the secondary coil to a vacuo-junction and measuring the heat produced by a tetanizing stimulus at various coil angles. The galvanometer deflection is proportional to the square of the current. The curve relating coil angle to current strength was similar to that previously published (Downing, Gerard and Hill, 1926). Third, the rheobase and chronaxie were determined in the usual manner, with the apparatus used by Boyd and Gerard (1930).

5. *Stimulation.* The threshold tests just described were made at infrequent intervals and for as short a time as possible, so that the nerve serving as a resting control had very little stimulation. The experimental nerve, on the other hand, was stimulated from almost at once after severing (within half an hour) by means of a rotating interruptor and inductorium. This gave a tetanus lasting 0.1 second repeated every 1.1 second, which stimulation was maintained continuously except at the times of testing for threshold, when it was momentarily interrupted. The strength of the current was made just maximal at the start and thereafter increased as necessary to continue to evoke the maximal contraction obtainable.

RESULTS. 1. *Muscular response to maximal stimulation.* The maximal muscular response obtained on stimulating either the peroneal or tibial nerve remained almost constant for 30 to 40 hours after nerve section, the strength of stimulus to the nerve being varied in each case until the maximal response was obtained. The height of contraction then dropped rather sharply in the next 10 or 15 hours to a small fraction of its original value, and then fell very slowly until at about 70 hours no further response could be obtained (fig. 3). Throughout, and for considerably after this time, the response obtained from direct stimulation of the muscle remained entirely constant, unless the electrodes in the muscle happened to be inserted near the point of entry of the nerve. In this case, at the time of nerve failure there was also some decrease in muscular response. This was undoubtedly due to the dropping out of muscle fibers originally indirectly

excited through the nerve, which was itself stimulated as it entered the muscle. It was regularly observed that when electrodes in the muscle were nearer together, the maximum response obtainable was less than when they were further apart. In other words, when fewer fibers were within the field of current flow, a lesser contraction was obtained. While the nerve still conducted, muscle fibers at a distance were also activated, but they dropped out when nerve conduction failed. These results refer to stimulation with a tetanizing current.

In terms of the all-or-nothing law, the obvious interpretation—of the gradual decrease in height of muscular response to a maximal stimulation of the nerve at a distance—is that individual nerve fibers progressively failed entirely to conduct, while those still functioning evoked a maximal contraction of the muscle fibers still innervated.¹ This interpretation is

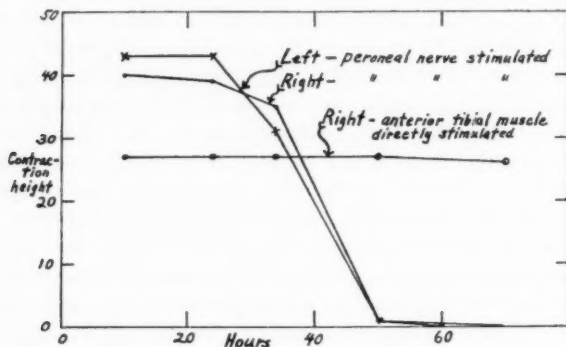


Fig. 3

confirmed by the studies on nerve threshold, changes in which do not closely parallel the changes in height of contraction.

In many control experiments in which both peroneals or both tibials were cut and neither stimulated, the times and manners of failure were practically identical, and such differences as appeared were equally distributed in favor of one side or the other (fig. 3). In all cases in which one nerve was stimulated and its opposite companion not, however, the re-

¹ It is now known, of course, that individual muscle fibers are able to respond in a graded fashion to graded stimuli, when these are very sharply localized. Normally, excitation via the nerve evokes only maximal responses; but the possibility must be considered that subnormal impulses conducted by degenerating fibers would lead to submaximal responses of the innervated muscle units. In the light of the findings of Gelfan and Gerard (1930), it seems most probable that, since the excitation coming via the nerve acts on the conductile mechanism of the muscle, even subnormal nerve impulses, if effective, will call forth maximal muscle responses.

sponse of the muscle on the stimulated side fell more rapidly and reached zero sooner than on the unstimulated side. The averages for all experiments are shown in figure 4. The intermittent activity of the severed nerve obviously, therefore, led to a more rapid failure of the fibers rather

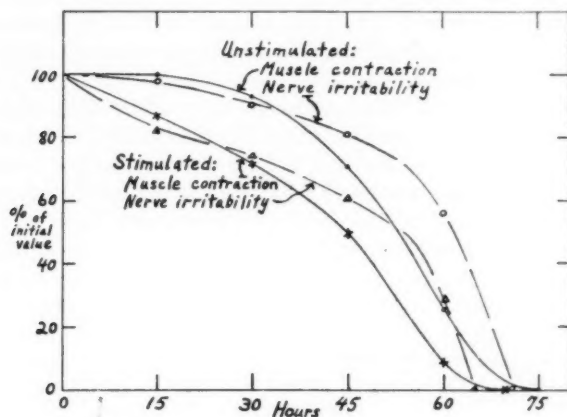


Fig. 4

TABLE 1

TIME AFTER SECTION	IRRITABILITY (REFERRED TO INITIAL VALUE)	
	Unstimulated (average of 8)	Stimulated (average of 6)
hours	per cent	per cent
0	100	100
15	99	81
30	90	74
45	81	61
60	58	29
Last response obtained at.....	67 hours	61 hours
Interval to next test (no response).....	3 hours	5 hours

than the reverse. The average time to complete failure was, for all peroneal nerves, 68 hours; for all tibials, 66.

2. *Nerve threshold.* The nerve irritability, as measured by the threshold primary resistance, fell slowly at first and then progressively more rapidly to a sharp inexcitability. The average curves of failure for 8 experiments without stimulation and 6 with are shown in figure 4, table 1. The irrita-

bility of the stimulated nerves falls distinctly more rapidly than that of the resting ones. Both average curves are slightly misleading in that a rather definite break in the individual ones tends to be smoothed out. Actually, in each case, the irritability falls relatively slowly for most of the period and then is rapidly lost. When the cored coil is used, a more marked progressive fall of irritability is observed, though here stimulation first delayed and then accelerated it. It is in harmony with the results obtained with condenser discharges, to be discussed at once, that the effectiveness of the more gradual shocks delivered by the cored coil decreases more markedly than that of the very sharp ones delivered by the coreless coil. Even here, however, a gradual threshold rise is terminated abruptly by complete inexcitability. This course is in harmony with the

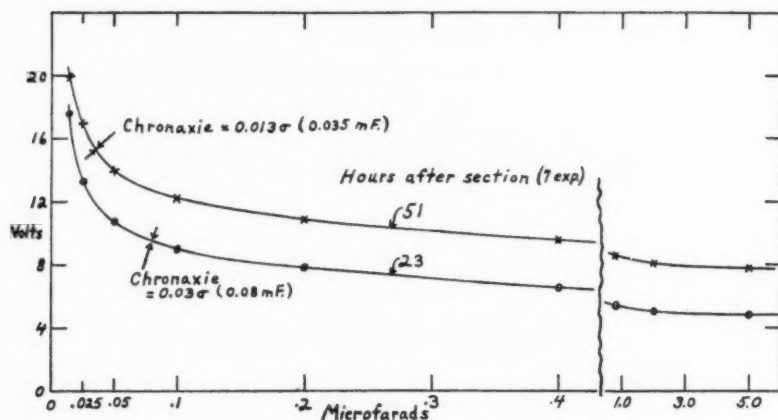


Fig. 5

view advanced that fibers fail in succession. For each individual case, degenerative changes, accompanied by a rising threshold, proceed to a critical stage, when block ensues. The now denervated muscle fibers then drop from the total of active ones.

3. *Chronaxie and intensity-time threshold curve.* For long enduring currents, determining essentially the rheobase, the threshold of practically all nerves studied rose progressively from the time of section until the death of the animal (fig. 5). This rise was usually quite marked, the average at two days being almost twice the initial value. With very short and intense currents, the change with time since section was much less striking. In several cases, the high-voltage, short-time limb of curves taken at different intervals after section coincided, although the long-time low-voltage portions were widely separated. The effect of such a change

in stimulation relations would, of course, lead to a shorter chronaxie. The chronaxie did, in fact, decrease under these conditions from an average value of 0.029σ at 23 hours to one of 0.015σ at 51 hours, while the rheobase rose from 4.9 to 8.3 volts during the same period. At 39 hours, however, the chronaxie and rheobase were both greater than earlier.

These findings are rather the reverse of what one might expect, in view of the reaction of degeneration of muscle. There very short, intense stimuli become non-effective before the long, weak ones, or, as ordinarily expressed, the muscle becomes inexcitable to the faradic current, whereas it retains its excitability to the galvanic. As regards stimulation, we have not sufficient comparative intensity-duration data to justify any analysis.

DISCUSSION. It seems obvious from the above that, under the conditions of stimulation here used, nerve fibers severed from their cells cannot be maintained in a functional condition. On the contrary, the evidence indicates that such stimulated fibers undergo more rapid loss of irritability and conductivity than the companion resting ones. Further, these axones become inactive long before the muscle fibers which they innervate have lost their ability to respond, in an approximately normal fashion, to direct stimulation. This evidence definitely favors the view that the integrity of the nerve fiber is dependent on some type of chemical substance spreading along it from the nerve cell, rather than on any type of transmitted activity. Spread might be by diffusion or more rapidly, as along interfaces.

It has several times been urged that a cut nerve degenerates centrifugally (e.g., Bethe, 1903). We are unable to find any evidence for centrifugal loss of conductivity. When two similar sets of electrodes are buried proximally and distally on the same cut nerve, the response fails and disappears simultaneously at both, unless the distal one is very close to the muscle. In this latter case, a similar decrease of response appears as with higher electrodes, but some slight twitches usually persist for a longer time. This is understandable in terms of the progressive loss of function of individual fibers.

During the two or three days following section, the height of muscle response obtained with a maximal stimulus falls progressively to zero, although the threshold of the nerve falls relatively little until the terminal inexcitability. This can be most simply explained by the complete falling out of individual neurones in succession, while those still conducting are able to evoke a practically normal response. The gradual rise in threshold prior to the final loss of conductivity near the end of the run might be due to the earlier failure of fibers of high threshold than those of low, or might represent a gradual true increase in threshold in all fibers. The slow rise in threshold over two or three days, followed by abrupt complete failure of the nerve would seem to suggest the latter rather than the former, as does also the relative change in threshold for currents of different durations.

This time change would hardly occur if those fibers still active had not changed. It is, of course, entirely possible or even likely that, in addition, the different fiber types degenerate at different rates, so that the larger, more excitable ones fail first. The fact that the nerve threshold changes little until the muscle response suddenly disappears, indicates also that the failure of muscle response is due to blocked conduction all along the axone, rather than an inexcitability limited to the electrode region. Obviously, if anywhere along the course of a single axone, conditions became such as to preclude conductivity, it would be completely blocked from above, and similarly, since there is no *a priori* reason for such a break occurring at one level more than another, for each fiber the block might first occur anywhere along its course. The nearer to the muscle, therefore, that the fiber is stimulated, the less chance of including the blocked region between stimulus and effector organ, and hence the possibility of still obtaining feeble muscle twitches on stimulating near the muscle when none can be obtained on stimulation at a greater distance.

The possibility must still be considered that failure of muscle response is due not to lack of conduction in the nerve, but to failure in the terminal end-organs. The above evidence is largely compatible with either possibility. In order to distinguish between the two, we dissected out the cut and uncut nerves from the two sides, in several cases immediately after conduction failed in the one, and tested a peripheral stretch of each for action currents. This was done on the apparatus previously used by Gerard (1930), and under these conditions the undegenerated nerve gave deflections of the galvanometer of 100 mm. or more. In no case did the degenerated nerve give more than 5 mm. deflection, and in many cases, none whatever. This indicates again that most or all fibers had completely lost conductivity at the time the muscle response could no longer be evoked.

We will not attempt any detailed comparison of the physiological and histological changes in the nerve after section, since we have no histological material of our own for comparison. There seems to be fairly general agreement, however, that the earliest histological changes, such as swelling, are seen in the axis cylinder 24 hours or less after section (e.g., Bucy, 1928) and are quite marked at two days; whereas the myelin sheath shows little change before the second to fourth day. Since block was complete in our experiments on the average at 67 hours, this further indicates the greater importance of the axis cylinder than the myelin in conduction. In any one degenerating nerve, the various individual fibers are rarely in the same state of degeneration, some being nearly completely fragmented, while adjoining ones may appear still normal. This is again consistent with our results and the interpretation that fibers fall out individually and successively. It would be of the greatest interest to be able to correlate failure of individual fibers with histological changes involving the neuro-fibrils

rather than the whole axis cylinder, but the data do not justify such a detailed discussion.

It remains to consider why a stimulated nerve should block and fail sooner than the unstimulated controls. If the inference previously drawn, that degeneration is due to the absence of substances reaching the fiber from the cell body, be correct, this further effect of stimulation would seem to follow naturally enough. It may be noted in passing that the more rapid degeneration on the stimulated side might be related to better blood flow due to muscular activity. Since the active muscles were, however, far peripheral to the major length of the nerve, this does not seem a likely reason, and a control experiment with nerves of both legs cut and the femoral artery on one side ligated in the groin showed no differences in the course of failure during 45 hours, when, unfortunately, the dog died. During activity, the metabolic changes in a nerve fiber are greatly increased over those at rest. More oxygen is consumed and more foodstuff burned with the production of carbon dioxide. None of these substances, however, can be deficient, since they are readily and continuously supplied by the blood-stream. Whatever is lacking must not reach the nerve fiber directly through the blood-stream; otherwise severing it from the cell body would be immaterial. In addition to oxygen and foodstuff, the oxidative processes of cells require the presence of catalytically active substances which are generally believed to be related to, possibly formed by, the nucleus. In nerve, the iron containing Nissl substance comes to mind; though its formation, fate and function are still largely conjectural. There is no reason to believe that such oxidizing enzymes would circulate in the blood, whereas they might diffuse down the nerve. If these enter into the oxidative reactions and are very slowly lost as a result of their participation, it would follow automatically that the stimulated fiber should lose its activity sooner than the resting one. Whether this particular suggestion prove valid or not, it remains very probably true that the increased metabolism of the active nerve exhausts sooner than otherwise a store of some substance necessary for its continued activity and even integrity.

It may be noted in this connection that frog nerve degenerates more rapidly at higher temperatures than low. The Q_{10} is 2.2 from 14°C.-24°C. (Rindovie, 1925), which is exactly that of the respiratory rate (Gerard, 1927).

We have described one type of experiment to determine whether axone integrity depends on impulses transmitted or substances spreading along it. When spreading is stopped by section and impulses artificially maintained, degeneration is not prevented. The reverse experiment would be equally interesting—to maintain the possibility of chemical spread while blocking conduction of impulses. It is a simple matter *in vitro* to block nerve conduction for hours, by electric currents, cold, pressure, etc. Simi-

lar treatment of an uninjured nerve *in vivo* might be expected to keep conduction in abeyance for an indefinite period without disturbing the nutritional situation. The nerve, then, should not degenerate, and when the block is subsequently lifted, functional activity might be present from the start. We have attempted such experiments on dog sciatics using electrotonic, pressure and cold blocks, but technical difficulties have so far prevented determinative results.

Fortunately, in lieu of laboratory experiments, there are numerous clinical data bearing on the problem of block and nerve function. Perhaps most striking are experiences with hypophyseal tumors. Dr. John F. Fulton has kindly put at our disposal the records of several such cases observed by him in Dr. Cushing's clinic. Most apropos is one of sub-total blindness of months' standing, due to pressure on the optic nerves by an hypophyseal adenoma, in which vision had improved remarkably by two to three hours after operative removal of the growth, and was close to normal when perimetry was performed six days later. Since no possibility of rapid, if any, regeneration exists under these conditions, the evidence clearly favors the continued integrity of nerve fibers severed functionally, but not structurally, from their cell bodies.

SUMMARY

A method of considerable range of usefulness for the continued stimulation of nerves *in situ* in living animals by means of buried electrodes is described.

It has been used in the present series of experiments on dog sciatics to determine the influence of such stimulation on the course of degeneration of the peripheral portion of a severed nerve.

The experiments show that a cut nerve loses its ability to conduct more rapidly when stimulated than when at rest.

Data on irritability and on muscle response lead to the conclusion that individual axones fail in temporal sequence. In each axone the threshold rises, and conduction is probably depressed, until it has passed a critical value at some point along the fiber, when conduction is blocked.

Degeneration of a nerve process isolated from its cell body might be due to lack of impulses conducted by it or of necessary substances spreading along it. The evidence considered favors the second possibility.

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SIMULTANEOUS STUDY OF THE CONSTITUENTS OF THE SWEAT, URINE AND BLOOD, ALSO GASTRIC ACIDITY AND OTHER MANIFESTATIONS RESULTING FROM SWEATING¹

VIII. BLOOD CHANGES

G. A. TALBERT, A. K. SAIKI, R. C. CARPENTER, J. BERGMAYER, H. STAFF,
C. BORMAN AND D. FREEMAN

From the Physiological Laboratory of the University of North Dakota

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This article deals with an extension of the work which was started some time ago for the purpose of obtaining more data on the changes that take place in the blood and urine as a result of profuse sweating.

There has been a considerable amount of work done in the past few years dealing with the effects of tub baths and their attending heat upon the blood with the use of both human and animal subjects. The reports from these observations have been more or less conflicting and thereby have arisen some controversial discussions.

One can quite readily imagine how some of these differences might arise, especially in the use of the human subject that sweats profusely compared with the practically non-sweating animal, such as the dog. In the former case we are dealing with a better heat regulation through the skin, while in the latter case, the lungs become more of a factor. Furthermore, the cabinet bath, due to factors of radiation and conduction, might yield important differences from the total immersion of the tub bath. This is particularly to be kept in mind when one knows that the blood and urine changes under such influences are more or less transitory.

In this article we are reporting results obtained after profuse sweating provoked by moist heat generated by electric current.

The subject was seated in the sweat cabinet with the head only protruding, while the arms and torso were completely enclosed in a rather thick rubber dam jacket with a belt around the waist. The jacket with the belt was for the primary purpose of collecting the sweat for other experiments not reported in this article. The jacket, however, served

¹ The expenses of this research were partially covered by four grants from the American Medical Association Research Fund.

a secondary purpose of provoking sweat more readily and it was a means of standardizing all of our experiments.

There were 54 different subjects used, of whom all were students except one who was a janitor. The subjects reported in the morning at six-thirty, eight, and ten o'clock respectively. Each one served as a rule three times a week.

The experiments herein cited have to do with bodily temperature changes, the fluctuations in the blood as to pH, specific gravity, and CO₂ combining power; also the changes in CO₂ of the expired air. The blood samples were taken just before entering and immediately after leaving the cabinet, while the temperature and expired air samples were taken just before entering and just before retiring from the cabinet.

The cabinet temperature usually registered 40 to 42 degrees Centigrade. All external air currents were carefully excluded. The subjects remained in the cabinet until sweating was profuse or extreme discomfort was felt.

The time varied from 20 to 50 minutes, depending much upon the external atmospheric temperature and humidity, as these experiments were performed in the summer.

Bodily temperature. The subjects experienced a rise in temperature varying from a few tenths up to three, and, even in rare cases, four degrees Fahrenheit. The greatest rise, as a rule, was in those who came at six-thirty. In many instances the control readings were surprisingly low, in some cases below 96°F. In some of these low control readings the subjects did not reach the normal temperature at the end of the experiments, while those of a higher control not infrequently registered a fever temperature at the end of the experiment.

Specific gravity. The specific gravity of the blood was obtained by the Barbour and Hamilton (1) falling drop method, which, with the proper control of the factors of error, seemed the most reliable method that could be used. Reports of several observers on the effect of heat on blood concentration have been conflicting. Barbour (2) with the same method claims that in his experiments on dogs he obtained a blood dilution which he maintains is in agreement with the older literature, including observations on man. However, in another article, Barbour and Tolstoi (3) state that in an environment of 42°C. dogs generally experience a rise in bodily temperature and a blood dilution. On the other hand, a sudden rise in bodily temperature was associated with a blood concentration.

Bazett (4) reports that in experiments upon himself in hot baths there was an increase in hemoglobin. However, in other subjects in neutral baths there was found a slight dilution followed by a concentration. He advances the tentative hypothesis that a drop in hemoglobin percentages occurs in the reaction to the rise in skin temperature produced by the bath which would bring the results into agreement with Barbour's,

and that this tendency is opposed and eventually overcome by some other action of the bath which causes the concentration.

On the contrary, Flinn and Scott (5) with dogs as subjects were unable at any time to note a blood dilution as a result of hot baths. Furthermore, they state that the total solids increases as the environmental temperature rises.

Gerhartz (6) found as a result of work there was a diminution of water in the blood and a consequent increase in specific gravity, hemoglobin and nitrogen. It is a fair assumption that in work the concentration might in a great measure be due to the sudden requisition of the excretory glands and more particularly the sweat glands.

Our results show most uniformly a blood concentration from the sweat cabinet experiments and thereby our report on human subjects is quite in harmony with that of Flinn and Scott. We have, it is true, found some

TABLE I

	FALL IN BLOOD CO ₂ COMBINING POWER	RISE IN BLOOD, pH	RISE IN BLOOD, SPECIFIC GRAVITY	PER CENT RISE IN CO ₂ OF EXPIRED AIR
Subject Br.....	1.8	0.00	0.0057	26.6
Subject C.....	7.8	0.03	0.0041	59.3
Subject D.....	2.1	0.04	0.0014	51.2
Subject Br.....	4.5	0.04	0.0036	35.9
Subject T.....	4.2	0.02	0.0035	20.5
Subject C.....	3.8	0.07	0.0003	8.1
Subject H.....	1.5	0.00	0.0001	2.6
Subject Bo.....	4.4	0.02	0.0019	43.6
Subject Ha.....	8.1	0.03	0.0027	47.7
Subject D.....	6.2	0.02	0.0020	24.3

exceptions, but out of 320 experiments we have discovered only 36 instances where there was not a concentration.

During the summer of 1930, we did 78 experiments in which 6 showed a blood dilution. Three of these were from a subject who was known to have done some manual work previous to the experiment which would invalidate somewhat the control sample. As to the other three, I will attempt no explanation.

In the face of what seems to us strong evidence, we are rather persuaded that the interpretation set forth by Flinn and Scott is the most convincing. We say this in due deference to the skin reaction and other possible factors of the tub bath as suggested by Bazett. In support of our views we would state that the amount of sweat secreted and the consequent loss in body weight in the short time of the experiment would in itself furnish consider-

able evidence that the rate of replacement of water cannot keep pace with the rate of loss. These results explain our blood concentration.

Some work has been done in the study of the relation of blood concentration to pathologic fever and it has become of much interest to what extent a similar correlation might obtain in physiologic fever. Lozinsky (13) states that dogs in moist air between 27° to 30°C. regulate body temperature by blood dilution and in moist temperature above 33° are unable to regulate body temperature and animals become practically poikilothermous and blood concentration takes place. May we not assume in a lesser degree the same obtains in the human subject, particularly in our experiments where the subject's body is pretty much enclosed in a rubber jacket thereby causing the dissipation of body heat to be less effective?

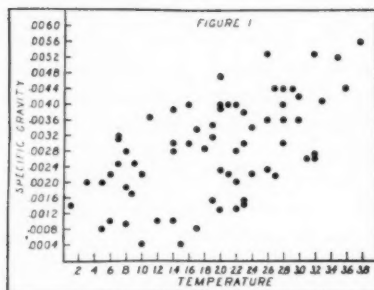


Fig. 1

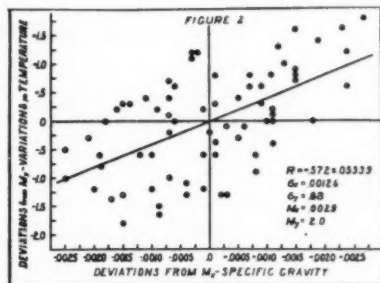


Fig. 2

In the 78 experiments performed in 1930 we checked the blood concentration with the temperature changes. In figure 1 we have plotted 72 of these experiments, omitting the six exceptional cases cited above. The actual rise in specific gravity is to be found on the ordinate and the temperature rise is recorded in tenths of a degree on the abscissa. From the same data the deviation from the mean is shown in figure 2 in which R gives a value of 57.2 which looks like a fairly good percentage of correlation.

CO₂ of blood. The CO₂ combining power of the plasma was determined by the Van Slyke (7) method. We have done in all 212 experiments of which 160 show a fall in CO₂ varying from a trifle to over 10 volumes per cent; more frequently 3 to 5 volumes per cent were recorded. In the experiments where there was a gain in CO₂, it was most frequently small. However in one instance over 3 volumes per cent was observed.

pH changes. The pH changes of the blood were determined by the well known Cullen and Hastings (8) colorimetric method, the solutions being

renewed twice a week. In 134 observations 110 show a rise in pH, 14 show a loss, and in 10 no changes were indicated. The changes were not marked; as a rule a variation of 0.01 to 0.07 of a pH was most common. During the summer of 1930 we were able to check the CO_2 combining power with the pH in 69 experiments. In 42 cases there was a gain in pH with a loss of CO_2 . In 5 cases a loss of pH and a gain in CO_2 . In 13 cases a gain in both and in 4 cases a loss in both.

Just to what extent we might be dealing with factors of digestion and morning alkaline tide is a matter of some conjecture. However, the quite general fall in CO_2 combining power and more general rise in pH seems to occur in spite of the time of day or the relation to digestion; consequently we are inclined to the belief that we are dealing with other attending and over-ruling factors.

A similar relationship between the pH and CO_2 combining power has previously been noted by other investigators. Haggard (9), for instance, states that "a rise in body temperature lowers the alveolar tension and reduces the amount of CO_2 in solution in the blood. Furthermore the alkali in use is not lowered in proportion to the decrease in dissolved CO_2 . The CO_2 is therefore reduced and the CH presumably lowered," which we found quite uniformly in our experiments.

Cajori, Crouter and Pemberton (10) claimed there was enough change in acid base equilibrium in quite mild exposure to heat to produce a measurable increase in alkalinity of the blood. Flinn and Scott, as well as Bazett and others, have emphasized that excessive ventilation with its consequent washing out of CO_2 would be sufficient cause for the rise in pH; Bazett and Haldane (11) have noted a urine alkaline wave resulting. The last named point is hardly in agreement with our experience for Talbert (12) reported some years ago and quite recently unpublished data by Bergmeyer and Staff of this laboratory have revealed more frequently a greater urine acidity, although at times alkaline tide has been noted.

As to the point of the alkalosis being caused by over-ventilation, we have had occasion to check that factor by use of the Newcomer modified Haldane apparatus. There were in all 110 experiments of this character which were done simultaneously with the taking of the pH, specific gravity and CO_2 combining power. In the table we have selected ten typical cases.

In conclusion, we wish to acknowledge that some of the data used in this article were obtained by A. Squires, R. Goehl, E. Fluvog, and we wish to thank Doctor Lavine of the Chemical Engineering Department for his assistance in pH determination. Most of all, we would acknowledge our indebtedness to Doctor Hektoen and his committee in giving us four grants from the American Medical Association Research Fund.

SUMMARY

1. There is a fairly good correlation between the rise in bodily temperature and the increase in blood concentration.

2. Under the conditions in which these experiments were performed we have found quite a uniform decrease in CO_2 combining power and a rise in pH of the blood.

3. Further evidence is offered that the rise of bodily temperature provokes greater ventilation and thereby increases the blood pH.

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THE UREA CLEARANCE TEST IN NORMAL DOGS

ELAINE P. RALLI, MARSHALL BROWN AND ARTHUR PARIENTE

From the Laboratories of the Department of Medicine, New York University

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The urea clearance test was done on a series of normal dogs to ascertain the average clearance in dogs. The test was carried out according to the method of Möller, McIntosh and Van Slyke (1). In this the standard blood urea clearance (Cs) represents the number of cubic centimeters of blood cleared of its urea content by one minute's excretion of urine. In normal human subjects Austin, Stillman and Van Slyke (2) have shown that with urine volumes below a certain point (2 cc. per minute) the urea output increases in direct proportion to the square root of the urine volume and that when urine volume reaches this limit urea excretion attains its maximum unaffected by further increase in urine volume. These observations were confirmed by Möller, McIntosh and Van Slyke (1) who established the relationship between urine volume and the rate of urea excretion for normal subjects. In figuring the clearance on normal dogs we have corrected the volume per minute by taking the square root of the minute output, thus calculating for a "standard clearance." The average minute output in the dogs studied was 0.19 cc., the extremes being 1.049 cc. and 0.025 cc. As the minute output was uniformly small we felt that the conditions of the "standard clearance" were established and therefore corrected the volume per minute by taking its square root.

EXPERIMENTAL PROCEDURE. Female dogs were used. The animals were kept under standard conditions on a mixed diet sufficient to maintain their body weight. In doing the clearance test the technique of Möller, McIntosh and Van Slyke (1) was followed. The experiments were performed in the morning, about seventeen hours after the last meal. The animal was catheterized, the specimen discarded and the dog returned to her cage. At intervals of one and two hours the dog was again catheterized. No water was given during the experimental period. A sample of blood was taken at the end of the first hour. The blood urea nitrogen was determined on this sample by the Van Slyke gasometric method (3). The urine urea nitrogen was determined on each specimen of urine by the same method. The clearance was figured separately for each hour and the average clearance is the average of these two hours.

RESULTS. The results of the urea clearance done on the ten dogs are shown in table 1. The clearances varied from 26.7 cc. to 113.2 cc. This variation bears a direct relation to the size of the dog. In order to reduce this to standard conditions for dogs of various sizes, the Cs on each dog was divided by the surface area of the dog. The surface area was calculated according to the formula of Cowgill and Drabkin (4). Taylor, Drury and Addis (5) found that their "urea excretion ratio" which is the "maximum clearance" as defined by Möller, McIntosh and Van Slyke (1) parallels more exactly the body surface than the body weight. This seems reasonable as Dreyer and his co-workers (6) found that blood volume parallels surface area. Furthermore, Taylor et al. (5) found that in rabbits the kidney weights vary in proportion to the surface area. These authors corrected their urea excretion ratios in patients by multiplying them by the factor

$$\frac{\text{average normal surface area}}{\text{area of subject}}$$

This work was later confirmed by McIntosh, Möller and Van Slyke (7). It is not possible to calculate the average surface area for laboratory dogs as they vary too greatly in size, nor does it seem necessary as by figuring the surface area according to the formula of Cowgill and Drabkin, and dividing the Cs by this, one obtains the number of cubic centimeters of blood cleared per square meter of body surface. Table 2 shows the results obtained by dividing the "standard clearance" by the surface area. We found that normal dogs have a Cs of 76.56 cc. to 121.36 cc. per square meter of body surface. The average Cs/S.A. was 99.46 cc. with a standard deviation of 21.9. According to Dunn (8) 66 per cent of all observations should fall within the range of the standard deviation and 95 and 99 per cent should fall within two and three times the standard deviation respectively. In this series of observations 66 per cent fall within the range of the standard deviation, 95 per cent and 100 per cent fall within the range of two and three times the standard deviation respectively. Dunn considers that no variation from the mean can be considered significant unless it is three times the standard deviation. Therefore for a clearance to be abnormal in a dog it would have to fall below 33.7 cc. per square meter of body surface. In none of these normal dogs did the Cs/S.A. fall below this figure.

In eight of the dogs, nos. 1, 3, 4, 5, 6, 7, 9 and 10, the kidneys were weighed. In these, the Cs divided by the kidney weight in grams gives an average of 0.7799 or an average Cs of 77.99 cc. per 100 grams of kidney substance in normal dogs. The extremes were 57.14 cc. to 110.5 cc. In dogs 1, 6

TABLE 1
Urea clearances on normal dogs

DOG NUMBER	DATE	WEIGHT kgm.	BLOOD UREA N mgm.	URINE UREA N		U/B RATIO		MINUTE VOLUME		STANDARD CLEARANCE (Cs)			AVERAGE OF CLEAR- ANCES
				First hour mgm.	Second hour mgm.	First hour	Second hour	First hour	Second hour	First hour	Second hour	Average	
1	9/26/29	9.0	15.7	1749.0	1880.0	111.4	119.7	0.146	0.119	42.53	41.18	41.855	41.90
	10/ 2/29	9.0	14.8	1563.0	1540.0	105.7	104.2	0.178	0.167	44.64	42.65	43.645	
	10/ 3/29	9.0	11.71	1142.0	1153.5	99.97	98.5	0.189	0.149	42.33	38.62	40.475	
2	9/23/29	11.5	18.16	2224.0	2233.0	122.5	123.0	0.256	0.226	55.82	55.66	55.74	71.22
	9/24/29	11.5	12.89	1270.0	1472.0	98.55	114.4	0.750	0.450	85.35	76.75	81.05	
	9/25/29	11.5	8.43	1365.0	1605.0	161.9	190.4	0.209	0.175	74.15	79.59	76.87	
3	10/22/29	7.0	20.58	1361.0	1374.9	66.2	66.8	0.286	0.254	35.5	33.8	34.65	36.64
	10/24/29	7.0	23.15	1579.0	1669.0	68.25	72.2	0.348	0.262	40.3	36.95	38.625	
4	10/15/29	10.0	18.89	1465.0	1475.0	77.6	78.2	0.307	0.226	43.0	37.2	40.1	42.975
	10/16/29	10.0	18.15	1021.0	1362.0	56.3	75.1	1.049	0.204	57.8	33.9	45.85	
5	11/ 6/29	9.75	23.05	1714.0	2350.0	71.6	98.2	0.369	0.197	43.4	43.6	43.5	49.9
	11/ 7/29	9.75	26.6	2360.0	2410.0	88.8	90.7	0.452	0.339	59.7	52.9	56.3	
6	10/18/29	7.5	15.73	2341.0	2710.0	148.9	172.0	0.032	0.025	26.35	27.05	26.7	30.0
	10/21/29	7.5	9.42	1825.0	1579.0	192.8	167.7	0.047	0.034	41.8	30.85	36.325	
	10/22/29	7.5	11.23	1392.0	1411.0	124.0	125.9	0.062	0.034	30.75	23.2	26.975	
7	2/ 7/30	12.75	18.2	2595.0	2694.0	142.5	147.7	0.100	0.085	45.1	43.0	44.05	49.24
	2/25/30	12.75	19.1	3380.0	3260.0	177.0	170.8	0.092	0.080	53.77	48.3	51.03	
	3/12/30	12.2	15.83	3160.0	2815.0	206.0	183.2	0.074	0.071	56.2	49.1	52.65	

8	4/24/30	19.5	13.7	2691.0	3146.0	196.5	230.7	0.203	0.169	88.53	94.43	91.48	102.39
	4/29/30	19.75	9.74	1985.5	1935.5	203.8	198.6	0.269	0.222	132.9	93.54	113.2	
9	9/17/30	10.5	21.18	2871.0	2587.0	135.53	122.13	0.266	0.267	70.03	63.23	66.63	
	9/26/30	10.5	12.7	1680.0	1696.0	132.3	133.6	0.123	0.114	46.41	45.3	45.855	54.82
	10/10/30	10.33	16.22	2548.0	2609.0	157.1	160.9	0.114	0.100	53.04	50.89	51.97	
10	10/28/30	10.0	12.62	2432.0	2984.0	192.76	236.56	0.083	0.073	55.55	64.23	59.89	
	10/31/30	10.33	11.29	1823.0	2170.0	161.5	192.25	0.129	0.092	58.00	58.21	58.11	63.09
	11/ 8/30	10.33	16.34	2505.0	2703.0	153.3	165.4	0.147	0.097	58.97	51.46	55.22	
	11/11/30	10.25	7.07	1662.0	1953.0	235.0	276.13	0.113	0.082	79.00	79.3	79.15	

TABLE 2

A comparison of the standard clearance (Cs), the standard clearance divided by the surface area (Cs/S.A.), and the standard clearance divided by the kidney weight in grams multiplied by 100 (Cs/Kd. Wt. \times 100)

DOG	S.A.	Cs.	Cs/S.A.	KIDNEY WEIGHT	Cs/Kd. Wt. \times 100	dog	S.A.	Cs.	Cs/S.A.	KIDNEY WEIGHT	Cs/Kd. Wt. \times 100
		cc.	cc.	grams	cc.			cc.	cc.	grams	cc.
1	0.57	43	75.47	57.5	75.44	6	0.38	26	68.51	42.0	61.9
		45	78.95		78.95			27	71.1		64.29
		42	73.68		75.40			42	110.5		100.0
		41	71.92		71.93			31	81.6		73.81
		43	75.47		75.44			31	81.6		73.81
		39	68.40		68.42			23	60.6		54.75
2	0.58	56	96.55	Kidney weight not known		7	0.55	45	81.8	57.7	76.21
		56	96.55					43	78.1		74.52
		85	146.3					54	98.2		93.59
		77	132.8					48	87.2		83.19
		74	127.9					56	101.8		97.05
		78	134.5					49	89.1		84.92
3	0.45	35	77.78	59.5	58.83	8	0.88	88	100.0	Dog alive	
		40	88.89		67.23			94	106.8		
		34	75.56		57.14			133	151.2		
		37	82.22		62.17			94	106.8		
4	*	43		52.5	81.91	9	0.54	70	129.5	69.0	101.0
		58			110.5			63	116.5		91.3
		47			89.52			46	85.1		66.6
		34			64.76			45	83.2		65.2
								53	98.0		76.8
								51	94.3		73.9
5	0.54	43	79.63	72.0	59.72	10	0.58	55	96.4	75.0	73.0
		60	111.1		83.33			64	110.2		85.0
		44	81.48		61.11			58	100.0		77.0
		53	98.15		73.61			58	100.0		77.0
								59	101.4		78.0
								51	88.6		68.0
								79	136.0		105.0
								79	136.0		105.0

* Unable to calculate surface areas as length of dog was not measured.

and 7 the average figure for the Cs/S.A. is remarkably close to that of the Cs/kidney weight times 100.

DOG	AV. Cs/S.A.	AV. Cs/K. Wt. X100
	cc.	cc.
1	73.95	74.26
3	81.11	61.34
5	92.59	69.44
6	78.95	71.26
7	89.3	84.97
9	101.1	79.1
10	108.6	83.5

It seemed to us that this should hold true for all of the dogs, if, as is true in rabbits, the surface area parallels the kidney weight. Possibly if more animals were studied this difference would be reduced, or it may be that as dogs of mixed breeds were used that the parallelism between surface area and kidney weight is not as close as in animals of one breed.

The excretion of urea nitrogen per hour was found to bear a definite relation to the urine volume per hour. With volumes below 10 cc. per hour the urea nitrogen rarely exceeded 200 mgm. With volumes ranging from 12 cc. to 30 cc. per hour the hourly excretion remained between 200 and 500 mgm. When the volume per hour rose above 40 cc., which might be considered a maximum urine volume for an average size dog, the urea nitrogen rose above 500 mgm. This is in accordance with Addis' observations (9).

SUMMARY

1. The urea clearance test was done according to the method of Möller, McIntosh and Van Slyke on ten normal female dogs.

2. The standard urea clearance varied according to the size of the dog so that it was not possible to arrive at a comparable figure for normal animals of varying sizes. However, when the standard clearance of each animal was divided by the animal's surface area, thus giving the results in cubic centimeter of blood cleared per square meter of body surface, the standard clearance for normal dogs was found to be 99.46 cc. per square meter of body surface with a deviation of 21.9.

3. In seven of the ten dogs the kidneys were weighed. The standard clearance per 100 grams of kidney substance averaged 76.88 cc.

4. In three of the seven dogs the kidney weight in grams paralleled the square meters of body surface.

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ADDENDUM

Since this article went to press, Dr. Norman Jolliffe and Dr. Homer Smith of the Department of Physiology, New York University, have found that according to observations made by them the augmentation limit in dogs, kept under the conditions of our experiment, is in the vicinity of 0.4 cc. per minute. Our figures substantiate these findings and we recognize that for those urine volumes in our data which are above this limit, the clearance should be treated as a linear function of the volume.

STUDIES ON HEART-BLOCK IN THE TERRAPIN¹

ARTHUR H. HURD

From the Department of Physiology, School of Medicine, The Johns Hopkins University

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The work of Erlanger (1) forms a classic demonstration of the fact that artificial heart-block is dependent upon and proportional to the clamp tension exerted upon the atrio-ventricular (His) bundle of the mammal. Subsequent experiments of Erlanger and Hirschfelder (2) demonstrated the lengthening of the As-Vs interval (in the mammal) preparatory to a missed beat or the establishment of halved rhythm (2:1), and thereby confirmed this lengthened As-Vs interval as one of the manifestations of deranged conduction. This fact has been observed repeatedly in clinical cases, and is tabulated by Lewis (3) as the lowest grade of heart-block. Still focusing attention upon the older literature, however, one sees that it is *not* a steadily progressive change which occurs in the bundle tissue while the grade of block is increasing, since further work of Erlanger and Hirschfelder (4) demonstrated the differential results obtained from vagal stimulation. They showed that partial block permitted vagal inhibition of mammalian ventricles to take place just as readily as when conduction was normal, whereas with complete block, as is known, the vagus exerts no negative chronotropic effect.

A. RELATION OF COMPENSATORY PAUSE TO THE DEGREE OF A-V CLAMP. It was with the aim of determining whether ventricular irritability could be affected by auriculo-ventricular block, in itself, that the first of this series of experiments was attempted. All of this and subsequent work was performed upon species of terrapin that could not be identified always with certainty.²

The plastron was removed and the pericardium opened in the usual manner, and threads were attached to the *frenulum cordis* and the left auricle, for the mechanical registration of ventricular and auricular beats, respectively. The apparatus used, similar in part to that employed in subsequent determinations, consisted of *a*, a Gaskell clamp, which was

¹ Acknowledgment is made to the Henry Strong Denison Medical Foundation, for a grant that assisted the author materially in carrying out this work.

² The specimens used were most probably *Malaclemmys centrata* (Latreille) subspecies *concentrica* (Shaw) and *Trachemys* (*Pseudemys*) *elegans* (Wild). One individual of *Pseudemys rubriventris* (Leconte) was also used.

made to grasp the A-V junctional tissue and hold it firmly, yet without excess pressure on adjacent structures; and *b*, inductorium, and a combination chronograph and marking key. Stimuli were applied to the ventricle by means of leads attached to apex and base. An additional induction coil and a pair of stimulating electrodes were employed for vagal stimulation.

The procedure consisted in applying induction shocks when the ventricle was as nearly in mid-diastole as possible, and noting the effect upon the compensatory pause as the Gaskell clamp was tightened. By selecting the above position in the cardiac cycle, the constantly increasing irritability of the ventricle tended to compensate for any variation in the time of applying the stimulus. For purposes of abbreviation the term EVs is applied to the artificially produced ventricular extrasystole, in contrast to the normal Vs.

Seven experiments in all were carried through and their graphic records analyzed. The accompanying protocol gives an example of the results of such analysis. A survey of all the experiments leads one to conclude that there is no definite lengthening of the compensatory pause, proportional to the amount of pressure on the conducting tissue, until the clamp is exerting a great deal of tension. Then there is a pronounced lengthening of the pause. Obviously, however, as one approaches a total block, the phenomenon of a predictable pause after EVs tends to vanish, and one can obtain any sort of reading that one wishes. Stimulation of the vagus tends to lengthen this pause, only when a definite slowing of the beat accompanies it, which is what one would expect as a result of the lengthened cycle.

Detail of experiment of April 29, 1930, recording observations of total pauses (EVs-Vs interval in seconds)

Without clamp tension:

No stimulation: 2.6, 3.1, 2.6, 3.2, 2.5, 3.3 seconds. Average of 2.88 seconds.

Right vagus stimulated: 2.6, 2.8, 3.5, 2.6 seconds. Average of 2.88 seconds.

Left vagus stimulated: 3.10, 2.30, 2.90, 3.10 seconds. Average of 2.85 seconds.

With clamp tension of one degree:

No stimulation: 2.5, 2.6, 2.5, 2.6, 2.7, 2.4, 2.5, 2.5 seconds. Average of 2.54 seconds.

Right vagus stimulated: 1.85, 2.15, 1.95, 1.75, 1.65, 1.75 seconds. Average of 1.85 seconds.

Left vagus stimulated: 1.90, 2.00, 1.80, 1.85 seconds. Average of 1.89 seconds.

With clamp tension of two degrees:

No stimulation: 2.15, 2.10, 1.95, 2.00 seconds. Average of 2.05 seconds.

Right vagus stimulated: 2.00, 1.85, 1.95, 1.75 seconds. Average of 1.89 seconds.

Left vagus stimulated: (a) 3.65 seconds, (b) 2.20, 3.10, 2.20 seconds. Average of 2.50 seconds.

With clamp tension of three degrees:

No stimulation: 1.85, 1.90, 1.90, 2.05, 1.85 seconds. Average of 1.91 seconds.

Right vagus stimulated: 2.20, 1.75, 1.90, 1.75, 1.85, 1.75 seconds. Average of 1.90 seconds.

Left vagus stimulated: 2.10, 1.90 seconds. Average of 2.00 seconds.

With clamp tension of four degrees:

No stimulation: 4.6 seconds.

With clamp tension of five degrees:

No stimulation: 5.6, 7.0 seconds. Average of 6.3 seconds.

With clamp tension of six degrees:

Right vagus stimulated: 6.1 seconds.

Substantially, increases in the measured pause occurred only when an "extra refractory period" was present, in which the ventricle failed to respond to one auricular beat. That no other constant lengthening occurred was corroborated by observing that the Vs following the EVs was identical with its successors in time relationships, thus proving that there was no mutually compensating change. The fact that a dropped beat thus followed a 1:1 ratio must be explained on the grounds that the induced systole was more complete than the normal ones (c.f. Howell's "dissociable material," as explained by Schultz (5)), and thereby created a prolongation of the refractory period; since with the heart in partial block (with 2:1 or 3:1 rhythm, etc.) results indicate that all the impulses to contract are propagated equally by the auricle, but that normal ventricular irritability does not recover sufficiently in one cycle to respond to these attenuated impulses (Erlanger, 6).

Further observations additionally corroborated this idea of a relative refractory period with constant occurring stimuli. By the simple procedure of obtaining a 2:1 or 3:1 rhythm ratio, by block, and stimulating the ventricle after it had been idle for one auricular cycle, for example, the ensuing EVs-Vs could be compared with the normal Vs-Vs interval. In most cases the two intervals were found to be essentially the same—i.e., with a 3:1 rhythm and a stimulus (induction shock) striking the ventricle during its idle period, this chamber tended to wait for just 3 auricular beats before responding again. Thus the time of the normal effective stimulus could be changed at will as demonstrated in figure 1. That there may be a decrease of irritability on the part of the ventricle, is shown by the fact that on two occasions a ventricle which previously had responded to either make or break stimuli, with an extreme degree of clamp now only responded to break stimuli.

The effect of the extra stimulus was sometimes to change the grade of block. In some cases it reduced it, and speeded up the ventricle—that is, a 2:1 ratio might be reduced to a 1:1; or if the latter already existed, the ventricle actually might appear to beat in 5:4 ratio with the auricle (probably the so-called reentry phenomenon of circus movement, ex-

plained by Schmitt and Erlanger (7)). On the other hand, the grade of block might be increased by the extra stimulus, usually so as to cause ventricular stasis. The latter, however, usually was not permanent, thus suggesting that the cause was merely a long recovery period following an over-violent stimulus.

B. LATENT PERIOD DETERMINATIONS. As a means of determining quantitatively the change in ventricular irritability which is known to occur between contractions (6) (8), it was decided to make observations as to the latent period between the time of stimulus and the systole, as may be obtained from drum records, while definite partial block was pres-

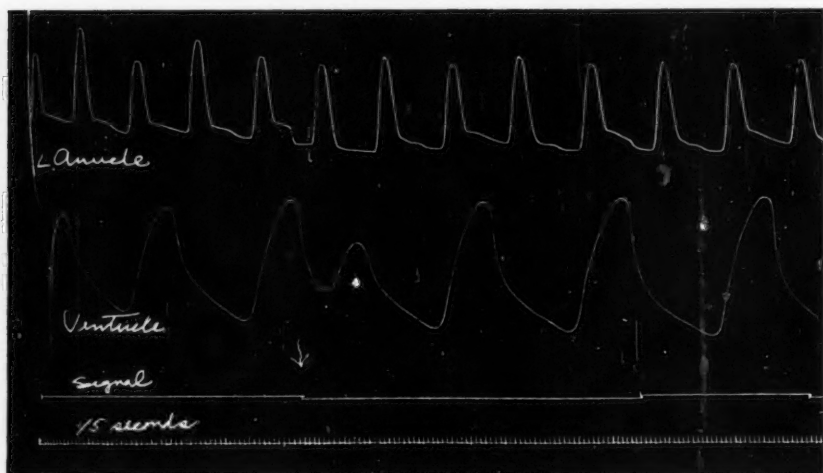


Fig. 1. Record of the heart beating in a condition of 2:1 block. The arrow indicates the point at which an electrical shock is applied to the ventricle. The EVs-Vs interval is seen to be substantially equal to the Vs-Vs one, and thus the time when an ineffectual stimulus for the ventricle occurs is permanently changed.

ent. Induction shocks were applied through electrodes attached to apex and base of ventricle, which, for purposes of convenience, were classed as "early" or "late" stimuli, depending upon their time of application during the elongated period of ventricular standstill. The latent periods were measured by means of a clock marking fifth-seconds, and a signal key automatically recording on a drum, which revolved fast enough to permit one to measure to 0.05 second.

Experiments were performed upon twelve hearts. The analysis of the graphic records (see addendum, table 2) and the tenor of their results are exemplified in the following summary of a typical experiment:

Protocol of determination of 9/19/30

TIME OF APPLYING STIMULUS	GRADE OF BLOCK	LATENT PERIOD READINGS (SECOND)	AVERAGE
Early	2:1	A. 0.35, 0.35, 0.40, 0.35, 0.40, 0.40 B. 0.30, 0.30, 0.40, 0.35, 0.35	0.36 second (early)
Early	2:1 and 3:1	C. 0.40, 0.35, 0.35, 0.35, 0.30	
Late	2:1	D. 0.25, 0.20, 0.20, 0.20, 0.20 E. 0.15, 0.20, 0.20, 0.15	0.21 second (late)
Late	3:1	F. 0.15, 0.20, 0.20, 0.20, 0.25 G. 0.25, 0.25, 0.20, 0.30	

One can thus observe quantitatively how a stimulus which reaches the ventricle during the earlier part of its rest period (with the heart in a condition of partial block) finds the chamber-wall in a much more refractory condition than does a stimulus which reaches the ventricle late in the rest period. In the case of perfusion with a solution high in KCl concentration, and of higher osmotic pressure, there appeared long latent periods and a great difference between "early" and "late" readings. This effect could not, however, be obtained with solutions high in KCl concentration but of normal osmotic pressure, which eliminates any possible effect of the K-ion. Successive stimuli applied at varying intervals during a condition of total block, and stimuli applied "early" while a 1:1 ratio existed, showed no consistent variation in results from those comparably applied while a partial block was affecting the ratio.

C. CHANGES IN THE AS-Vs INTERVAL. Along with the data on nine of the hearts just considered, there are also data on the change which occurred in the interval between auricular and ventricular systoles (As-Vs).

The time interval is measured, first immediately following vagal inhibition, and again after the establishment of a partial or complete block and recovery to a 1:1 rhythm. The latter condition was always sought for, so as to eliminate the increase in irritability which would result from a long ventricular pause.

The measurements show the following for the As-Vs interval (in seconds) before establishing partial block as compared with the "early" and "late" As-Vs interval in seconds after establishing the partial block. The second decimal place is only approximately correct: 0.30 → 0.75 and 0.75; 0.45 → 0.65 and 0.90; 0.40 → 0.50 and 0.90; 0.25 → 0.90 and 1.15; 0.50 → 1.80 and > 1.80; 1.20 → 2.80 and 2.80. When the block was total, the figures are: 0.80 → 1.50, and then 1.30 with 2:1 ratio, in Ringer's; 0.90 → 1.60 in Ringer's and then to 1.40 in 0.1 per cent KCl; 0.80 → 1.40 in Ringer's and then to 1.10 in 0.1 per cent and 0.15 per cent KCl.

The right vagus was stimulated in four of these hearts and in every

case the initial As-Vs interval was shortened, thus: $0.8 \rightarrow 0.5$; $1.2 \rightarrow 1.1$; $0.9 \rightarrow 0.8$; $0.8 \rightarrow 0.6$ second. The left vagus was stimulated in one case only and this increased the As-Vs interval from 0.5 to 0.9 second.

From these measurements it becomes obvious that any clamp pressure sufficient to cause a partial or total block, even if the 1:1 ratio between heart-chamber beats is restored subsequently, has caused sufficient damage to the bundle tissue to delay conduction thereafter. Engelmann (8) has mentioned a number of things (increase in normal auricular frequency, rapid loss of blood, increased number of artificial or effective stimuli in a given time) which lengthen the As-Vs interval in the frog. All these things concern a lengthened refractory condition of the ventricle, and this lengthening is analogous to Lewis' low grade of block (3), indicating alteration of, or damage to, the conducting tissue.

The degree of return to a normal As-Vs interval seems to be a function both of the individual tissue and of the initial clamp tension. However, it is safe to say that partial block due to clamp tension (sufficient to affect rhythm) is almost never followed by the return of an As-Vs interval which approaches normal. The effect of an excess of KCl to promote this return may be explained according to the results of Kirsch and Fredericq (9) who showed that either an excess of K^+ or a decrease of Ca^{++} in the perfusate would cause a decrease in chronaxie of the isolated tortoise heart. The effect of stimulating the *right* vagus, apparently to *increase irritability*, corresponds to the observation of Lapicque and Veil, as quoted by Evans (10), who found that the ventricular chronaxie was reduced during vagal stimulation. The effect of the *left* vagus, to *increase the As-Vs interval*, however, is what one would expect, if one assumes that the distribution of the left nerve is primarily to the A-V node, in contrast to the right vagus' distribution to the S-A node. This difference is manifest in the relative chronotropic effects produced by stimulating the two nerves, as observed by Garrey (11).

D. EFFECT OF VAGAL STIMULATION UPON DEGREE OF A-V BLOCK. In view of the known differences between the action of the two vagi on the hearts of certain terrapins (12) it was decided to determine the relative effectiveness of the two nerves in "breaking" a block, and to attempt to correlate these findings with their normal action. Erlanger and Hirschfelder (4) in 1906 stated that vagal stimulation removed a partial block in the dog's heart, but the chronotropic effect (slowing), in itself, obviously would increase the responsiveness of the ventricle. In the case of *total* block, involving ventricular standstill in the terrapin, this chronotropic effect could be eliminated; therefore faradization of the vagus was tried in several such cases. The normal effect of a tetanizing current of given strength was observed at the start of several experiments; subsequently, when partial or total block had been established, a given current again was applied to each nerve.

Out of 7 cases of partial block right vagus nerve stimulation reduced the degree of block 6 times. Out of 13 cases of complete block the reduction occurred 7 times. In the one case (of the 7 of partial block) where the vagal stimulation might have increased the block, the stimulation had no effect whatever.

In 4 cases out of 5 of partial block where the left vagus nerve was stimulated there was no effect whatever, and the one exceptional case showed only a slight increase in grade of block. In 3 cases of 1:1 rhythm left vagus stimulation produced partial block in one case, and total block in a second case. Out of 9 cases of total block left vagus stimulation produced a reduction of block in one case only.

From this analysis it is clear that the tendency of faradization of right vagus nerve is to relieve block, of left vagus nerve to produce block or increase grade of block. The latter effect is less certain than the former.

In accordance with Garrey's observations (11) these results would indicate that stimulation of the right vagus nerve is the more efficacious in producing a reduction in the grade of block. As an example of the latter, while the 1:1 ratio was being maintained, by bathing with 0.07 per cent KCl + 0.7 per cent NaCl solution, left vagal stimulation broke the rhythm and eventually established a total block, just as observed by Garrey (13), and this effect could be repeated at will. This result, together with the aforementioned right vagus effects, would indicate that in the majority of cases the left vagus and the right vagus are not strictly synergists, insofar as efficacy in removing heart block is concerned, just as again Garrey (12) showed a qualitative difference in the chronotropic effect of the two vagi in the terrapin. That a total block could be reduced by vagal stimulation indicates a positive bathmotropic effect upon the ventricle (apart from the slowing effect) as observed by Fredericq and Garrey (14), who obtained chronaxie readings of auricle and ventricle during vagal stimulation, while they simultaneously maintained constant rhythm in the turtle's heart by stimulation of the sinus venosus.

It is interesting to note that on two occasions, following vagal stimulation to remove a total block, the ventricle awakened before the auricle recommenced to beat. This is best explained as Engelmann (8) did in one of his cases: namely, that in the "water-logging" of a heart structure such as the auricle, the latter loses the properties of a muscle before it loses those of a nerve; it can conduct impulses from their origin to the ventricle tissue, while it actually cannot itself contract. Here again there seems to be conduction of an excitation wave without contraction in the (auricular) muscle.

E. EFFECT OF CHANGING THE CONCENTRATION OF ENVIRONMENTAL KCl AND CaCl_2 . In view of the effects of a moderate excess of K^+ on the heart, the well-known increase of inter-systolic relaxation and decrease in fre-

quency of the beat (analogous to vagus action as described by Botazzi, Howell (15)), and of the more recent observation of a temporary reduction in chronaxie by Kirsch and Fredericq (9), modified Ringer's solutions were employed with the object of reducing a given grade of block. In each case the potassium concentration was increased (thereby increasing the osmotic pressure) but in only one case was the calcium eliminated. In the earlier experiments the whole heart isolated from its own circulation was perfused *in situ* with an inflow cannula inserted in the left pre-cava, the outflow leading from the median aortic arch. But the occasional necessity for maintenance of a clamp around the A-V junctional tissue to preserve the grade of block, making the ventricular discharge of the perfusate uncertain, led to the mere bathing of the heart with the desired fluid in the last experiment. The opened pericardial space formed an admirable cup, and change of solutions was effected by drainage. Analysis of the results from the employment of the perfusion method (14 experiments on 6 terrapins) leads one to conclude that the increased irritability following perfusion with an excess of potassium is such as to reduce the grade of block, both as concerns the ratio of beats and as concerns conduction time, measured by the As-Vs interval. (According to Lewis and Master (16), both are different degrees of deficiency in the "decremental" conduction of impulses, which latter exists at all times.) With CaCl_2 retained in the solution, 0.10 per cent KCl appears to be about the optimum concentration for "breaking" a block, without at the same time causing too great a tonus deficit.

The final experiment was conducted merely by bathing the heart with the different solutions, which were rendered more nearly isotonic by eliminating CaCl_2 from those in which excess KCl was present; correspondingly, the required amount of potassium could be reduced to 0.04 per cent and 0.07 per cent. The protocol follows:

PROCEDURE	CHANGE
Clamped the heart while in Ringer's	A total block was <i>just</i> produced
Changed to 0.04% KCl and 0.7% NaCl	Block reduced, to alt. 3:1 and 5:1
Changed to 0.07% KCl and 0.7% NaCl	3:1 ratio established
Changed to normal Ringer's solution	At first no change; then 5:1, 6:1 and finally total block
Changed to 0.04% KCl and 0.7% NaCl	3:1 ratio established
Changed to 0.07% KCl and 0.7% NaCl	2:1 and 3:1; rhythm alternate
Changed to normal Ringer's solution	Practically total block established (1:10, 1:12)
Right vagal stimulation	Established 1:1 ratio, but long As-Vs interval
Changed to 0.07% KCl and 0.7% NaCl	Maintained 1:1 ratio
Left vagal stimulation	Broke the rhythm, and eventually established total block

These results make it apparent that a K^+ excess and right vagal stimulation may have synergistic action to increase ventricular irritability and thus reduce the grade of block, in contrast to a relative K^+ deficit and left vagal stimulation, which tend to increase the grade of block.

It was suspected, however, that in these experiments with the heart lying *in situ* an interfering effect might be produced by the ions which diffused from the surrounding tissues. Therefore, further experiments were performed, using isolated heart-vagus preparations from four terrapins of the same species. The intact heart, with both vagus nerves dissected free and attached, in each case was placed in a Petri dish, so that the solution employed could be changed or the nerves stimulated at will. A clamp supported by a T-arm grasped the aortic arches, thus forming resistance sufficient so that auricular and ventricular beats could be recorded mechanically. The effect of stimulating either nerve could be observed in all cases except the first; in this experiment, the right vagus was so damaged in the operation as to be useless.

In each case the results were essentially similar. No attempt will be made to incorporate these individual protocols in this paper, since for the most part they resemble preceding ones, but certain general observations will be summarized:

a. Initial stimulation of the left vagus (with the heart in Ringer's solution) almost invariably produced an elongation of the As-Vs interval, usually accompanied by a slowing of the rate; thus indicating two diametrically opposite effects. Corresponding stimulation of the right vagus tends to reduce the As-Vs interval, generally with a reduction in rate.

b. That the effect of the left vagus when stimulated (to lengthen the As-Vs interval) is due to a depression of the A-V conduction tissue, was demonstrated by means of mechanical stimulation. With the heart in a condition of total block in the last two experiments, a slight touch applied to the A-V junctional tissue elicited a ventricular response, whereas during left vagal stimulation the tissue seemed to become almost completely refractory to stimuli.

c. Similarly, increasing the KCl concentration nearly always shortened the As-Vs interval, whether or not an actual slowing in rate was concerned. After prolonged immersion, however, the interval tended to return to normal, as Kirsch and Fredericq (9) showed in the reverse way by using an excess of $CaCl_2$ in the solution perfusing the isolated heart of a tortoise. (In our cases the change was apparent after 5-15 minutes.)

d. In all these cases, either right vagal stimulation or an increase in the K^+ concentration frequently reduced the grade of either a total or a partial block, whereas left vagal stimulation or a relative decrease in the potassium present tended to increase the grade of any block which might be present. Upon occasions, a reduction in the grade of block might take place (through

either of the above methods), or conversely the grade of block might be increased, without the occurrence of any significant change in heart rate. This would indicate a definite separation of *chronotropic* and *bathmotropic* effects, and (as previously mentioned in connection with vagal stimulation) the frequent elimination of a total block indicates the same thing. Since there is no idio-ventricular rhythm in the terrapin's heart, any procedure (such as right vagal stimulation) which tends to remove a total block must act so as to affect ventricular irritability directly, and not as a function of a diminished rate (with longer "recovery" periods, between beats).

In discussing the possible causes which lead to the removal of block when the vagus trunk is stimulated faradically Garrey (17) has suggested, among other possible contributing causes, stimulation of sympathetic fibers that probably run along with the vagus sympathetic fibers in the cervical portion of the nerve. Izquierdo (18) on this suggestion has stimulated the (fusiform) inferior cervical sympathetic ganglion in several species of turtle and obtained results which support the idea that removal of auriculo-ventricular block is a function of sympathetic, rather than para-sympathetic, nerve stimulation. This view is not supported by the foregoing facts in this paper, and my own actual repetition of Izquierdo's experiment, stimulating the fusiform ganglion, has not thus far succeeded in reducing block. Why there is this discrepancy in our results is not yet clear. At the suggestion of Prof. C. D. Snyder the author is now at work on a pharmacological attack on the problem, which also, up to the time of this writing, not only fails to confirm the sympathetic nerve explanation but rather confirms the view that vagal stimulation removes block through impulses set up in the parasympathetic (preganglionic) fibers. A report of the results of this latter study is reserved for a separate paper.

SUMMARY

The foregoing observations on the heart of species of terrapin may be summarized as follows:

1. *a.* As the degree of A-V clamp tension is increased, there is no substantial lengthening in the compensatory pause of the terrapin's ventricle (following an external stimulus) until the clamp is exerting sufficient pressure to cause a single dropped beat, subsequent to the extra systole.

- b.* With 2:1, 3:1 or 4:1 rhythm, on the other hand, following such an artificial extrasystole there tends to be a ventricular pause of approximately the same length as the previous Vs-Vs interval. This confirms Erlanger's view that it is a variation in irritability of the ventricle, rather than a difference in strength of successive impulses, which determines the ventricular response in a condition of partial block.

- c.* Observation of latent periods, following induction shocks applied

to the ventricle during a condition of partial block, demonstrated quantitatively the above change in irritability.

2. The right vagus nerve, normally having the greatest negative chronotropic effect upon the heart, likewise is more efficacious in reducing either a partial or a total block, whereas the left vagus tends to increase the grade of block, confirming Garrey's analysis (1912).

3. Following any grade of block due to clamp, and the subsequent restoration of a 1:1 heart ratio, there is always an increase in the As-Vs interval. During perfusion with excess KCl or following right vagal stimulation there is a decrease, whereas following left vagal stimulation there tends to be an increase in the As-Vs interval.

4. About 0.10 per cent KCl with Ca^{++} in the Ringer's solution, or 0.07 per cent KCl in the absence of the calcium, used for perfusion or bathing, form the optimum concentrations to produce reduction of a partial block.

5. The effects of vagal stimulation and of alteration in KCl (in the bathing solution) were confirmed by observations made upon the isolated terrapin heart; likewise, one observed the dissociation of effects upon irritability from those upon rhythm. The immersed heart showed a tendency to adapt itself to fluid hypertonic in KCl.

The author wishes to express his most sincere appreciation of valuable suggestions and assistance rendered him by Prof. C. D. Snyder throughout this work.

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FURTHER STUDIES ON THE COMPOSITION OF SALIVA IN DIFFERENT PHASES OF THE SECRETION

HAMILTON BAXTER

From the Department of Physiology, McGill University, Montreal, Canada

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It was shown in a previous paper (Baxter, 1929) that the composition of saliva from a dog with a permanent parotid fistula changes in different phases of the secretory period. The percentage of organic matter obtained by different stimuli was higher in the second minute, i.e., during the initial period of the after-flow, than in the secretion collected during the first minute, when the animal was stimulated by food or certain rejectable substances. The organic and inorganic content of the after-flow of the succeeding four minutes was greatly reduced. Experiments on a second dog ("dog 2": the dog described in the first paper will be called "dog 1") showed a variation in some cases from the rise of organic matter in the second phase of the parotid secretion described above. The rise of organic matter in the second phase was often insignificant, and sometimes even lacking, depending on the stimulant.

In an effort to clarify this phenomenon the four-phase experiment was evolved; that is, the first phase was separated into two 30-second periods and each sample was determined separately. The phases were designated as 1(A), 1(B), 2 and 3. Phases 1(A) and 1(B) are of course merely equal divisions of phase 1 (during which the stimulus acted on the oral cavity, and which was described under three phase experiments). This arrangement permitted us to demonstrate the phenomenon described in dog 1 in all the animals employed in this investigation, and also to investigate the composition of the mixed glands. Later on, when sufficient data had been accumulated to render apparent the general laws governing phase action, the experiment was organized in a different fashion. The secretion during each of the four phases was collected, but instead of separate analyses being made, the samples from the four stimulations in each phase were mixed to form one uniform sample, which could be examined for chlorine as well as organic substances and ash.

Data relating to the composition of the saliva (organic matter, ash and chlorides) of the parotid and mixed glands during the four phases under different experimental conditions will be presented in this paper.

METHODS. Experiments were performed on five dogs with permanent salivary fistulae (after Gliniski). Two dogs (dog 1 and dog 3) had each a single parotid fistula; one dog (dog 2) had a single submaxillary fistula and one parotid fistula; and one (dog 4) had two parotid fistulae. The saliva was collected in graduated cylinders according to the generally used Pavlov method.

Great care was taken to obtain as many correct data as possible concerning the content of organic matter and ash in the saliva. Only special wide, shallow, platinum crucibles of 2 cc. capacity were employed in this investigation. The drying of the saliva and combustion of the organic matter was conducted in a special electric furnace described in a previous paper (Baxter, 1929). The time of heating necessary varies in direct proportion to the amount of organic substance present. If the platinum crucibles are placed in the furnace when it is cool, 30 to 45 minutes at the maximum temperature will generally be sufficient to remove all carbon.

As stated before (Baxter, 1929), the first sample of saliva collected after rest gave a greater content of organic substance than the following samples. Special experiments were performed to determine the influence of the interval between two stimulations on the composition of the saliva secreted. It was found that, when 30 minutes elapsed between two stimuli, the organic fraction was about twice the amount secreted when the interval was only 10 or 15 minutes. Our observations, together with those of Stepanoff (1921), resulted in the choice of a 15 minute time interval. Each dog was stimulated at 15 minute intervals not more than four times during an experiment, so that the animals were neither restless nor were the salivary glands exhausted by repeated stimulation.

Four phase experiments on the parotid gland. Table 1 shows the results of four phase experiments on the parotid gland in dogs 2, 3 and 4 after one minute's stimulation with bread and meat powder, 25 per cent HCl or 10 per cent NaCl. The amounts of saliva in table 1 represent the total volume of saliva in each phase, secreted during the four consecutive stimulations. The greatest concentration of organic substances in the parotid saliva was attained either in phase 1(B) or phase 2. Another interesting feature is that in phase 1(A) the secretion was almost invariably lower in both organic and ash fractions than in phase 1(B), although the amount of the secretion might be greater, equal or less. The average concentration of the organic moiety in phases 1(A) and 1(B) was usually lower than in phase 2.

There is evidence in this collection of data for the assertion that different dogs have individual peculiarities of response to stimulation, so that in one dog the organic matter may reach a peak in phase 1(B), while in another the greatest quantity will be present in phase 2, as it was in dog 1 (Baxter, 1929). However, in all the dogs with parotid fistula which were investigated by us, the secretion of organic matter rose to a maximum in phase 1(B) or 2 and then fell to a minimum in phase 3. Conditioned reflexes were excluded as far as possible before stimulation and in phase 3. Some conditioned influences, however, might play a part in the secretion of phase 3.

Another interesting deduction which may be made is that the difference

in the concentration of organic matter in the parotid saliva seen in phases 1(A) and 1(B), and phase 2, diminished with repeated stimulation.

Phases of secretion in the mixed glands. Experiments were performed on a dog (no. 2) with fistulae of the mixed glands and the parotid gland. The mixed glands also exhibit phases of secretion, but they are somewhat different from those of the parotid gland. The ash percentage was

TABLE 1

B. AND M. POWDER, MARCH 26						25 PER CENT HCl, MARCH 18						10 PER CENT NaCl, MARCH 20					
Phase	Amount	Solids	Organic material	Ash	Cl per 100 cc. of saliva	Phase	Amount	Solids	Organic material	Ash	Cl per 100 cc. of saliva	Phase	Amount	Solids	Organic material	Ash	Cl per 100 cc. of saliva
Parotid saliva: Dog 3																	
	cc.	per cent	per cent	per cent	mgm.		cc.	per cent	per cent	per cent	mgm.		cc.	per cent	per cent	per cent	mgm.
1A	6.1	1.34	0.62	0.72	278	1A	6.1	1.49	0.73	0.76	324	1A	5.2	1.10	0.41	0.69	306
1B	5.6	1.44	0.70	0.74	244	1B	5.4	1.96	1.15	0.81	292	1B	4.7	1.16	0.40	0.76	296
2	4.7	1.22	0.58	0.64	204	2	5.4	1.45	0.75	0.70	239	2	4.7	1.04	0.38	0.66	258
3	3.8	0.62	0.20	0.42	116	3	4.8	0.47	0.09	0.38	128	3	1.7	0.58	0.20	0.38	128
Parotid saliva: Dog 4																	
B. AND M. POWDER, MARCH 5						25 PER CENT HCl, MARCH 17						10 PER CENT NaCl, MARCH 17					
1A	3.3	1.10	0.28	0.82	350	1A	3.2	1.30	0.42	0.88	383	1A	4.85	1.09	0.34	0.75	328
1B	3.9	1.26	0.44	0.82	306	1B	4.75	1.70	0.81	0.89	340	1B	5.35	1.28	0.45	0.83	295
2	4.55	1.16	0.36	0.80	272	2	4.5	1.66	0.84	0.82	301	2	6.4	1.33	0.54	0.79	260
3	3.05	0.76	0.14	0.62	223	3	2.05	0.83	0.20	0.63	235	3	2.3	0.74	0.16	0.58	176
Saliva from mixed glands: Dog 2																	
B. AND M. POWDER, APRIL 2						25 PER CENT HCl, APRIL 1						10 PER CENT NaCl, APRIL 7					
1A	5.6	1.36	0.80	0.56	247	1A	6.2	1.22	0.54	0.68	238	1A	7.8	1.09	0.42	0.67	303
1B	5.6	1.38	0.84	0.54	221	1B	4.9	0.96	0.46	0.50	231	1B	6.9	1.04	0.39	0.65	302
2	4.8	1.42	0.88	0.54	166	2	4.2	0.92	0.48	0.44	176	2	8.1	0.96	0.38	0.58	250
3	3.9	0.96	0.68	0.28	58	3	1.8	0.88	0.52	0.36	142	3	5.2	0.65	0.32	0.33	123

usually lower in saliva from the mixed glands of this animal than in the saliva from the parotid gland. The secretion from the mixed glands, however, was always more copious than the secretion from the parotid gland in this dog. The percentage of organic matter did not undergo so much variation in the saliva of the mixed glands as in the parotid saliva, and usually fell from phase 1(A) to phase 2. In phase 3 there was little or no fall, and sometimes there was a rise above the level of the other phases.

Chlorine content of saliva from the parotid and mixed glands. In saliva both from the parotid and mixed glands, the chlorine (determined by Wilson and Bald's method) diminishes steadily from phase 1(A) to phase 3 independently of the concentration of the organic fraction and ash and of the rate of secretion. (See table 1.)

Since the organic content of the saliva may increase in phase 1(B) and 2, while the chlorine concentration falls, it may be assumed that the concentration of chlorine has no direct relation to the amount of organic matter secreted in the saliva. In general, the chlorine concentration of the parotid and mixed saliva is the higher, the greater the content of ash in the saliva. However, variations in the amount of chlorine run to a certain degree independently from the total weight of inorganic salts. This is true also for the variations of chlorine in each single experiment with all the different stimuli. Obviously some of the other inorganic salts present in saliva must vary in amount in each phase.

Effect of pilocarpine. The amount of pilocarpine hydrochloride which was injected subcutaneously was proportional to the size of the dog, and varied in amount from 2.5 to 3 mgm. The dose was purposely rather small, so that the whole course of secretion could be studied. Strict precautions were taken to avoid as much as possible the action of conditioned stimuli on the dog before and during the pilocarpine secretion. As was the rule, only a few drops of saliva were lost before the experiment started. The saliva was collected from the gland during 5-minute periods, and the content of solids, organic material and ash analyzed in each sample whenever it was possible. The secretion commenced in 5 minutes, reaching a maximum in 15 to 20 minutes, and then steadily declined. The whole process normally consumed approximately one hour.

The concentration of organic material in the first 5-minute period varied from 0.30 to 0.50 per cent, but rapidly declined during successive periods of the experiment, the content of organic substances in some experiments reaching as low a level as 0.10 per cent to 0.08 per cent. A slight increase sometimes occurred toward the end of the secretory period. The ash of the different samples pursues almost the same course as the organic fraction, falling to about 0.30 per cent. There are individual variations in the content of organic substances and ash, both in different dogs and in different experiments on the same dog, but the general course of secretion and the change in amount of its different constituents were practically the same.

The decreased concentration of the organic fraction of the saliva in consecutive samples cannot be explained by exhaustion of organic substance accumulated in the gland during rest, because when the mouth cavity was stimulated by food or hydrochloric acid during one 5-minute period of the pilocarpine secretion, there was always a great increase in

the amount of the organic moiety (table 2). The effect of this reflex stimulation was seen in the next two 5-minute periods. Analogous results were obtained when conditioned stimulation (food, HCl) was employed during the course of the pilocarpine secretion. Therefore we must regard the stimulation of the salivary gland by pilocarpine as a factor which does not excite its tropic function.

This explanation cannot be applied to the phenomenon of the diminished concentration of the inorganic constituents in pilocarpine saliva. According to the modern conception the inorganic substances in saliva are chiefly derived from the blood; only an insignificant amount may be present in the organic substances produced by the secretory cells themselves. Thus,

TABLE 2
Dog 2, 3 mgm. pilocarpine, February 20

TIME	PAROTID GLAND				MIXED GLANDS			
	Amount	Solids	Organic material	Ash	Amount	Solids	Organic material	Ash
	cc.	per cent	per cent	per cent	cc.	per cent	per cent	per cent
9:00	1.0	0.53	0.12	0.41	1.4	0.64	0.30	0.34
9:05	0.8				1.2			
9:10	0.8							
9:15	0.6	0.42	0.10	0.32	1.5	0.66	0.40	0.26
9:20	4.8*	0.90	0.37	0.53	9.7*	1.40	0.84	0.56
9:25	1.2	0.77	0.28	0.49	2.4	1.60	1.10	0.50
9:30	0.7	0.63	0.17	0.46	1.1	1.08	0.68	0.40
9:35	0.6	0.61	0.16	0.45	0.8	0.96	0.64	0.32
9:40	0.6				0.8			
9:45	0.5				0.7			

* Bread and meat powder was given to the dog for 10 seconds at the beginning of each minute of this 5-minute period.

with the increased flow of saliva during the later stages of the pilocarpine secretion, one might expect an increased concentration of salts. Our data show that the secretion of salts cannot be looked on as a mere filtration of inorganic constituents from the blood, but is a far more complicated process.

Exactly the same relations were observed in the course of the chlorine concentration during pilocarpine secretion. The concentration of chlorine rose when the salivary gland was stimulated reflexly (ingestion of bread and meat powder, 0.25 per cent HCl, etc.) during this secretion. Here again not only the immediate effect but also the after effect could be observed. Thus in one experiment (March 19), before the ingestion of meat and bread

powder, pilocarpine parotid secretion in one 5-minute period gave 1.5 cc. of saliva with the unusually low value of 135 mgm. per cent of Cl; during the following 5 minutes, when food was ingested, it gave 7.1 cc. with 296 mgm. per cent Cl; and during the next five minutes 1.8 cc. with 216 mgm. per cent Cl.

Effect of atropine. The data presented in the previous section show that the different constituents of the saliva produced by the gland may run to a certain degree independently of each other. Atropine was chosen as a drug affecting different parts of the neuro-cellular apparatus. Certain indications of the action of atropine in this respect were given in the work of Keeton, Luckhardt and Koch (1920) on gastric secretion.

The present experiments were performed on four normal dogs with parotid fistulae. The administration of atropine sulphate always took place half an hour before the first stimulation. Table 3 is a typical example of the effect of gradually increasing doses of atropine on the parotid gland of dog 1 in three-phase experiments, with 0.25 per cent hydrochloric acid as a stimulus. (The first line in the table represents the average figures calculated from a typical normal experiment with HCl.) Briefly, the effect of the drug in increasing amounts was a reduction in the percentage of ash and abolition of the phase rise in the organic moiety. Absolutely no effect was produced by the injection of 0.04 mgm. of atropine, while almost complete inhibition resulted from a dose of 4 mgm.

The increase in the percentage of organic substances in the saliva secreted when the gland was under the influence of atropine might be explained by the greater concentration of saliva due to the diminished secretion of water by the gland. However, the total output of organic substances was greatly diminished in the first two phases, and that of the ash in all three phases of the secretion. Also, the output of the organic fraction was decidedly increased in the third phase, when 0.8 mgm. and 2 mgm. of atropine were given (see table 3, showing total output of organic and inorganic substances calculated in milligrams). It seems that atropine in moderate doses affected both functions of the gland, the secretory (i.e., the secretion of water and salts) and the trophic function (i.e., liberation of organic substances from the cells) as may be seen from a study of the three phases.

CONCLUSION. The salivary glands of the dog respond to different stimuli with secretions differing in quantity and quality, not only during the action of the stimulus, as was previously known, but also in the various phases of the afterflow. The afterflow of the saliva from the parotid glands runs an independent course from that of the mixed glands, and the variations of the respective components of these secretions are different. The complicated character of the work of the salivary glands was specially emphasized in the experiments with pilocarpine and atropine. The pilocar-

TABLE 3
Dog 1 (weight 24 kg.)

PROCEDURE	SAMPLE	FIRST PHASE				SECOND PHASE				THIRD PHASE			
		Amount	Solids	Organic material	Ash	Amount	Solids	Organic material	Ash	Amount	Solids	Organic material	Ash
		cc.	per cent	per cent	per cent	cc.	per cent	per cent	per cent	cc.	per cent	per cent	per cent
0.25 per cent HCl	Average	2.8	1.36	0.56	0.81	1.5	1.74	0.91	0.83	1.1	0.74	0.14	0.60
Total output of organic and inorganic substances in milligram.....													
0.8 mgm. atropine sulphate and 0.25 per cent HCl October 23	1	1.4	1.25	0.46	0.79	0.6	1.58	0.58	0.73	0.1	1.45	0.97	0.48
	2	1.6	1.41	0.65	0.76	0.8	1.88	1.16	0.72	0.5	1.34	0.78	0.56
	3	1.8	1.38	0.61	0.77	0.8	1.57	0.83	0.74	0.6	1.14	0.64	0.50
	4	2.3	1.27	0.49	0.78	1.0	1.44	0.66	0.78	0.6	1.30	0.79	0.51
	Average	1.8	1.33	0.55	0.78	0.8	1.01	0.87	0.74	0.45	1.30	0.79	0.51
Total output of organic and inorganic substances in milligram.....													
2 mgm. atropine sulphate and 0.25 per cent HCl October 25	1	0.7	1.17	0.50	0.67	0.3	1.78	1.23	0.55	0.05	1.68	1.24	0.44
	2	0.7	1.61	0.97	0.64	0.3	1.54	0.92	0.62	0.3	1.16	0.70	0.46
	3	1.0	1.53	0.83	0.70	0.5	1.56	0.87	0.69	0.5	1.44	0.99	0.45
	4	1.0	1.50	0.78	0.72	0.7	1.71	1.00	0.71	0.3	4.32	2.97	1.35
	Average	0.8	1.46	0.77	0.69	0.45	7.69	4.50	3.19	0.3	4.32	2.97	1.35
Total output of organic and inorganic substances in milligram.....													
Total output of organic and inorganic substances in milligram.....	0.8	11.68	6.16	5.52	0.45	7.69	4.50	3.19	0.3	4.32	2.97	1.35	0.45
	0.8	11.68	6.16	5.52	0.45	7.69	4.50	3.19	0.3	4.32	2.97	1.35	0.45

4 mgm. atropine sulphate and 0.25 per cent HCl October 30	1	0.1																	
	2	0.1																	
	3	0.1																	
	4	0.4																	
	5	0.4																	
Average		0.2	1.68	1.17	0.51	0.15	1.66	1.21	0.45	0.04	1.45	1.15	0.30						
Total output of organic and inorganic substances in milligram.....		0.2	3.36	2.34	1.02	0.15	1.51	0.82	0.69	0.04	0.58	0.46	0.12						

pine experiments showed a steady fall in the concentration of the organic moiety of the saliva from the parotid and mixed glands, and also of the ash, the latter in spite of the greater volume of the secretion in some of the samples. On the other hand, a normal reflex stimulus from the mouth cavity greatly increased the concentration both of the organic and inorganic substances during the reflex action and its after-effect. Since during the artificial (electric) stimulation of the chorda tympani, the strengthening of the stimulus increased the outflow of organic and inorganic compounds in an unexhausted gland, pilocarpine cannot be considered as an exact equivalent of parasympathetic stimulation. The experiments with atropine revealed the fact that small doses of this drug diminish the secretion of water and inorganic substances in all three phases. The secretion of organic substances was inhibited in the first and second phases and greatly increased in the third phase, when the dose of atropine was not so large.

SUMMARY

1. The four-phase type of experiment reveals the fact that the salivary secretion of the parotid and mixed glands is composed of qualitatively and quantitatively different samples when the secretion of the various time periods is analyzed.

2. The chlorine concentration in saliva from the parotid and mixed glands diminishes steadily from phase 1(A) to phase 3. The concentration of chlorine has no relation to the amount of organic matter secreted.

3. In pilocarpine saliva, notwithstanding the increased secretion, there is invariably a fall in the organic and inorganic moieties, which cannot be attributed to exhaustion of the available supply. Therefore, the secretion of salts in the saliva under the influence of pilocarpine is not dependent only on the rate of secretion.

4. Atropine injected in progressively greater amounts diminishes the total output of fluid parts and of ash in all three phases of salivary secretion. In the third phase, however, it increased the total output of organic substances (diminished during the first and second phases), when the dose of atropine injected did not exceed 2 mgm.

The writer wishes to thank Dr. B. P. Babkin, under whose direction this investigation was carried out, for his very helpful advice and criticism.

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EXPERIMENTAL STUDIES ON THE EXTERNAL SECRETION OF THE PANCREAS WITH SPECIAL REFERENCE TO THE EFFECT OF ITS COMPLETE LOSS BY PERMANENT PANCREATIC FISTULA

I. THE COINCIDENT CHANGES IN THE CHEMISTRY OF THE BLOOD

II. THE MECHANISM OF DEATH¹

JOHN M. McCAUGHAN

From The Mayo Foundation, Rochester, Minnesota

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I. THE COINCIDENT CHANGES IN THE CHEMISTRY OF THE BLOOD. Removal of the pancreas from an animal is not necessarily fatal since the essential hormone of the pancreas can be supplied in the form of insulin. The exclusion of the pancreatic juice from the intestine by section and evulsion of its ducts, on the other hand, leads only to progressive loss of exocrine function with corresponding disturbances in protein and fat metabolism (Pratt, 1916; Pratt, Lamson and Marks, 1909). If, however, the entire output of the external secretion is excluded from the intestine by total drainage by fistula to the outside of the body, death invariably results in from five to eight days, with marked metabolic disturbances (Elman and McCaughan, 1926, 1927).

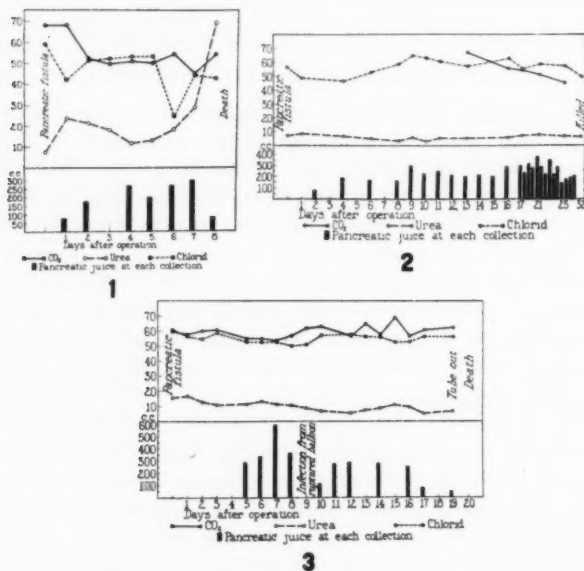
This study deals with other aspects of the total loss of pancreatic juice in dogs by means of a permanent pancreatic fistula, that is, the effect on the chemistry of the blood and the mechanism of death. Walters and Bollman, in 1927, using a more indirect method (acute duodenal fistula), likewise concluded that the loss of pancreatic secretion for any length of time was incompatible with life, whereas the loss of bile and duodenal secretion was without lethal effect.

EXPERIMENTAL PROCEDURES. Under ether anesthesia, with special regard to asepsis, the minor pancreatic duct was cut and evulsed. The major duct was then exposed and a suitable glass cannula attached to a long U-shaped rubber drainage tube inserted. The tube was next brought out through the flank, and connected with a glass T-tube, at one end of which a rubber balloon of a capacity of 300 to 500 cc. was attached. The collecting system was prepared beforehand as previously described. The blood plasma was analyzed daily for carbon dioxide, urea, and chlorides.

¹ Work done in the Division of Experimental Surgery and Pathology, The Mayo Foundation, Rochester, Minnesota.

RESULTS. The results previously noted (Elman and McCaughan, 1926, 1927) followed total drainage only; if drainage was less than total, untoward effects were not noted. Such subtotal drainage, however, served as a suitable control, as did other experiments in which there was no drainage. Occasionally an experiment was complicated by infection of the pancreatic juice or by some other operative or postoperative accident. For convenience, the experiments are grouped as follows: 1, total drainage; 2, subtotal drainage; and 3, infection of the pancreatic juice.

Total drainage. A pancreatic fistula was made on a dog weighing 15.9



kgm. Death occurred after eight days of total drainage (fig. 1). At necropsy cause for death other than the fistula could not be found. The chloride lost to the body in pancreatic juice amounted to 6.19 grams for the total period.

Subtotal drainage. A pancreatic fistula was made on a dog weighing 15 kgm. The animal after thirty-two days was in excellent condition (fig. 2). He was then killed by ether inhalation and examined for accessory ducts. A small duct was found which communicated with the duodenum.

A pancreatic fistula was made on a dog weighing 12.2 kgm. The animal was in excellent condition. The blood was normal. He was killed on the

fourteenth day and examined for accessory channels. It was possible to demonstrate a patent communication.

Infection of the pancreatic juice. Operation was performed on a dog weighing 6.8 kgm. (fig. 3). The juice became infected with *Bacillus coli*. On the nineteenth day after operation the animal died. Post-mortem examination was negative except for marked chronic pancreatitis. Accessory ducts could not be entirely excluded.

COMMENT. The total loss of pancreatic juice was invariably fatal in from five to eight days. A uniform syndrome was exhibited after operation, characterized by pronounced dehydration, weakness, anorexia, vomiting and death. The length of life showed no constant relationship to the weight of the animal, the quantity of juice lost or the rate at which it was secreted. The pulse, temperature, respirations and function of the kidney were not altered. Examination after death was invariably negative except for mild chronic inflammatory and degenerative changes in the pancreas.

It is to be emphasized that the results noted in the first experiment follow total drainage only. At times, however, considerable juice was lost over relatively long periods without untoward effects. In several such experiments extra ducts were demonstrated after death.

Pancreatic juice readily becomes infected, and the usual contaminants could be recovered by bacteriologic methods. The animals frequently manifested surprisingly little reaction.

The associated changes in the chemistry of the blood were noteworthy. The comparatively slight alteration in the blood chemistry gave little evidence of the profound general disturbance noted. This is interesting in view of the striking disturbances noted in high intestinal obstruction, anhydremia, and gastric or duodenal fistula (Marriott, 1923; Rowntree, 1922; Cooper, 1928; Walters and Bollman, 1926, 1927).

The fatal drainage of pancreatic juice was not attended with significant variations in the carbon dioxide combining power or chlorides.

The blood urea nitrogen, however, constantly showed moderate elevations, beginning as a rule several days preceding death. Evidence of renal impairment as measured by the output of phenolsulphonephthalein was lacking.

CONCLUSIONS. 1. The total drainage of pancreatic juice by fistula is rapidly fatal.

2. Subtotal drainage of pancreatic juice is without lethal effect within the period of observation.

3. Total drainage by fistula produces characteristic metabolic alterations. Anhydremia is to be emphasized particularly. The changes in the chemistry of the blood are chiefly marked by moderate elevations of urea preceding death.

II. MECHANISM OF DEATH. In order to throw some light on the prob-

able mechanism of death in cases of draining total pancreatic fistulas, it appeared likely that a study of the effects produced by the quantitative restoration of certain constituents of the pancreatic juice (alkaline fluid, sodium chloride and protein) might yield valuable data.

The effect of deprivation of chloride on the animal body is well known (Copher and Brooks, 1923; Dixon, 1924; Haden and Orr, 1923; Hartwell and Hogue, 1912; Trusler, 1928). In the external drainage of the pancreatic juice, however, not only sodium and chloride ions but bicarbonate are removed from the blood. Normally, of course, these substances are reabsorbed from the intestinal tract.

In pancreatic fistula the loss of chloride in the pancreatic juice (6 to 7 gm. in five to eight days) is not great.

The mode of death in experimental pancreatic fistula bears some resemblance to that of high intestinal obstruction. Differences of opinion exist as to the mechanism of death in intestinal obstruction, but most experimenters emphasize the early death, the rapid rise in the hydrogen-ion concentration and urea nitrogen of the blood and particularly the marked decrease in the blood chlorides.

In animals with pancreatic fistulas, rapidly progressive dehydration was noted. Anhydremia is known to alter the composition of the blood and lead to a fatal termination (Keith, 1922; Marriott, 1923; Rowntree, 1922).

Pancreatic juice is a significant factor in the regulation of gastric acidity (Apperly, 1926; Boldyreff, 1915; Iwanow, 1926; Olch, 1928). The absence of this regulating mechanism tends toward hypersecretion of the pancreatic juice by way of the fistula and the rapid depletion of minerals, protein and water is thus explained.

Disturbance in intestinal motility might be an additional factor of some importance as Walters and Bollman (1927) found in their work on acute duodenal fistula.

Certain coagulable proteins are present in fairly large amounts in pancreatic juice from the fistula. Little is known regarding their chemical nature or purpose but they undoubtedly are reabsorbed from the intestine under normal conditions.

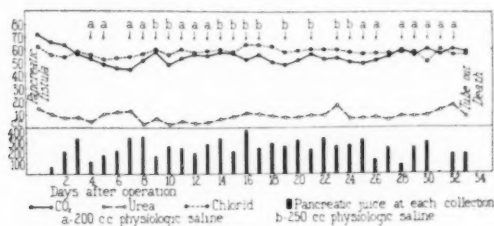
EXPERIMENTAL PROCEDURE. A closed type of pancreatic fistula preparation, described in previous papers (Elman and McCaughan, 1926, 1927) was used. Samples of blood were collected and analyzed for carbon dioxide combining power, urea nitrogen and sodium chloride. Gross and microscopic examination was made of all animals that died or were killed.

In the following experiments various constituents of the material lost from total pancreatic fistula preparations have been replaced in order to determine the effect, if any, on the duration of life, and on the chemistry of the blood. The experiments were as follows: 1, the effect of the intravenous injection of physiologic sodium chloride solution, and 2, the effect

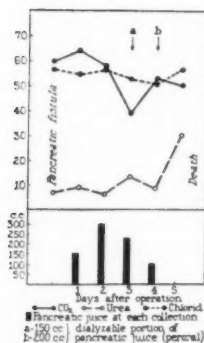
of administering either by mouth or vein whole pancreatic juice, a dialyzable portion, and a nondialyzable portion of the whole juice.

Intravenous administration of sodium chloride. Pancreatic fistula was made on a dog weighing 15.5 kgm. The juice became infected the fourteenth day and the animal died the thirty-fourth day after operation. Physiologic sodium chloride solution was given practically every day. The chemistry of the blood was not affected (fig. 4). On post-mortem examination cause for death other than fistula could not be found.

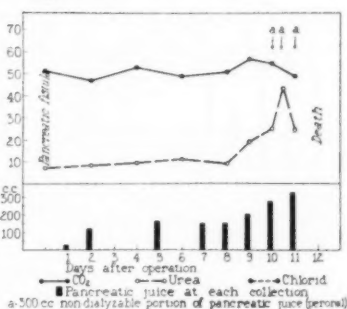
Oral administration of the dialyzable (crystalloid) fraction of pancreatic juice. Pancreatic fistula was made on a dog weighing 7.4 kgm. The ani-



4



5



6

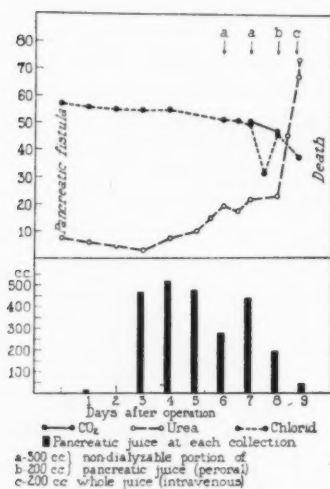
mal died on the fifth day after operation. The dialyzable juice given orally had no apparent influence on the chemistry of the blood or the duration of life in the amounts given (fig. 5). Post-mortem examination was entirely negative except for the fistula.

Oral administration of the non-dialyzable (colloid) fraction of the pancreatic juice. A pancreatic fistula was made on a dog weighing 22.3 kgm. The non-dialyzable portion did not have a definite effect, although the elevation of the blood urea might have been lessened (fig. 6).

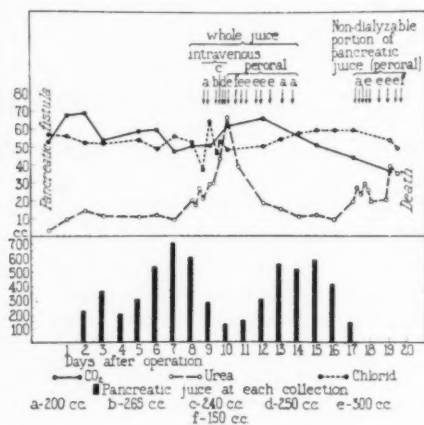
Death occurred on the twelfth day. Post-mortem examination was negative.

Oral administration of the nondialyzable fraction of the pancreatic juice and intravenous administration of whole pancreatic juice. A pancreatic fistula was made on a dog weighing 15.3 kgm. The non-dialyzable fraction of juice was given by mouth as soon as the animal's condition gave evidence of total fistula effect (fig. 7). On the ninth day 200 cc. of whole juice were given intravenously to a practically moribund animal, but death ensued before the injection could be repeated. At post-mortem examination cause for death other than total fistula could not be found.

Oral and intravenous administration of whole pancreatic juice. A pancreatic fistula was made on a dog weighing 14.1 kgm. The fifth day after



7



8

operation whole juice intravenously was begun (fig. 8). The animal rapidly grew worse. The use of the whole juice by mouth then produced a spectacular change within a few hours. The experiment was repeated subsequently with the use of the non-dialyzable portion. The animal died. The results of post-mortem examination were entirely negative.

COMMENT. It is shown that the administration of physiologic sodium chloride solution intravenously prolongs life and maintains normal blood.

The addition of sodium chloride and fluid must have been significant in controlling the deleterious effects of anhydremia. Pancreatic juice, as has been stated, contains considerable protein. The function of such proteins is unknown, but it is possible that their steady drain brings about

some imbalance in the colloid equilibrium of the blood and that the rise in blood urea nitrogen (retention) is an attempt by the body to combat this threatened disturbance of its colloid equilibrium. It is unlikely that the exclusion of pancreatic enzymes from the intestine for such short periods of time could be harmful; on the other hand, disturbances in the normal hydrogen-ion concentration of the duodenal juice with its effect on gastric acidity and motility are no doubt factors of some importance. It is recognized, however, that all factors may play a part: the loss of protein, water and salts in the juice. The end result may well be a summation of all the factors enumerated.

The effect of replacing certain portions separated from the juice by dialysis yielded unexpected results. The dialyzable and non-dialyzable portion given orally had no recognizable influence on either the chemistry of the blood or the post-operative course. Whole juice intravenously likewise had no demonstrable effect, whereas whole juice by mouth brought about notable improvement in the animal's condition, restored the chemistry of the blood to normal and prolonged life. This had been noted before (Elman and McCaughan, 1926).

Since the final preparation of this paper, Elman and Hartman (1930) have concluded from similar experiments that dehydration with resulting changes in circulation is probably the main factor in the rapid death. They also noted recovery and prolongation of life during drainage by the administration of sodium chloride, potassium chloride, and calcium chloride.

SUMMARY AND CONCLUSIONS

1. A moribund dog with total pancreatic fistula can be restored apparently to a normal state by oral administration of whole pancreatic juice; intravenous administration does not have such an effect.

2. The administration intravenously of physiologic sodium chloride solution daily during the period of total drainage will prolong life and maintain normal chemistry of the blood.

3. The administration of the dialyzable and non-dialyzable fractions of whole pancreatic juice was of no appreciable benefit as a life-saving measure in the amounts given.

4. The essential cause of early death following total drainage of pancreatic juice is not entirely clear. In general, the loss of salts, water and proteins by way of the pancreatic juice and gastric vomitus must play an important rôle. However, the comparatively slight disturbances in blood chemistry and the improvement in the condition of a moribund dog after oral administration of whole pancreatic juice leave much to be explained.

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THE EFFECT OF EPINEPHRINE ON MUSCLE AND LIVER GLYCOGEN

JACOB SACKS

From the Department of Physiology and Pharmacology, Northwestern University Medical School

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In 1928 Cori and Cori (2) reported that the injection of 0.2 mgm. of epinephrine per kilo body weight into fasted rats caused a decrease in the muscle glycogen and an increase in the liver glycogen. Eadie (6) reported two experiments on amyotized cats in which he found that 1.0 mgm. per kilo reduced the liver glycogen almost to zero. One cat showed no change in the glycogen content of its muscles, the other showed a decrease. Later (7) he reported other experiments with varying doses of epinephrine.

The present work was undertaken to study the effect of epinephrine on the muscle and liver glycogen of the 24-hour fasted cat. Two factors were varied: dosage and time. The doses used were 0.1 mgm. and 1.0 mgm. per kilo. The times were $1\frac{1}{2}$ and 3 hours. The experimental method will be given in detail so that it may serve as a basis for the discussion of the results obtained.

The animal was given an intraperitoneal injection of amytal, 75 mgm. per kilo, or nembutal, 45 mgm. per kilo. As soon as surgical anesthesia was obtained, one gastrocnemius was exposed and dissected free from surrounding structures. Care was taken not to interfere with its circulation or innervation. The skin incision was closed with clamps and the muscle allowed to recover from the trauma incident to the dissection. Meantime the femoral artery and vein were exposed high in the thigh and a ligature passed under them but not tied. An upper abdominal incision was then made. Fifteen minutes later the liver was brought out, a piece snipped off and dropped into a dish containing a slush of carbon dioxide snow and ether. The tissue was completely frozen within fifteen seconds after its removal from the animal. The raw surface of the liver was seared with a hot file to stop bleeding. The prepared muscle was then frozen. This was done by placing carbon dioxide snow around the tendon of insertion, which was cut at this time, and pouring on ether until a slush formed. This slush was worked toward the origin, thus freezing the muscle and its circulation simultaneously. This is the method described by Davenport and Davenport (5) with ether substituted for ethyl chloride at the suggestion of Doctor

Davenport. The time required for freezing the muscle in this way is under one minute. There is usually only slight momentary twitching, and by working from insertion to origin one avoids stimulation of the unfrozen muscle. The ligature around the femoral vessels was pulled tight and tied, the frozen muscle removed, the epinephrine solution injected subcutaneously, and the leg amputated at the knee. For the large dose, stock 1:1000 solution of epinephrine was used; for the small dose, freshly prepared 1:10,000 solution. The control animals were treated the same way except for the epinephrine injection.

After suturing the skin incisions, the animal was warmed up to its original body temperature and an additional equal amount of the anesthetic originally used was administered subcutaneously. By this means complete surgical anesthesia was maintained for the duration of the experiment. Either $1\frac{1}{2}$ or 3 hours after the epinephrine injection, or after the removal of the first samples in the case of the controls, the remaining gastrocnemius and another portion of the liver were frozen in the same way.

The frozen tissues were scraped free from blood and each portion sliced into a dry beaker surrounded by carbon dioxide snow. About a gram of sliced tissue was transferred to a tared centrifuge tube and rapidly weighed on a torsion balance to within 5 mgm. Then 5 cc. of 30 per cent KOH solution were added and the tube plunged into a boiling water bath. From this point the usual Pflüger technique was followed. The final glycogen precipitates were hydrolyzed with 0.5 N HCl for $2\frac{1}{2}$ to 3 hours at the temperature of boiling water. All the samples from one cat were hydrolyzed simultaneously. Glucose was determined by the Somogyi modification of the Shaffer-Hartmann method. Glycogen is given in terms of glucose found.

RESULTS. *Control animals:* In table 1 it will be noted that there are appreciable differences in the glycogen content of the two muscles of a control animal, but that the average for the group shows practically no loss over a period of three hours. In regard to the liver glycogen: there is no appreciable loss in $1\frac{1}{2}$ hours, as compared with the initial values, but there is an average decrease of $\frac{1}{2}$ the total amount at the end of 3 hours.

Animals receiving 0.1 mgm. of epinephrine per kilo: The muscles show no change; the livers show a definite decrease. In the $1\frac{1}{2}$ hours group the average loss is 19.5 per cent, compared to 2 per cent in the controls. In the 3 hour group, the difference is less striking; it amounts to 35 per cent against 25 per cent in the controls. This may mean that the peak of the epinephrine effect has been passed.

Animals receiving 1.0 mgm. per kilo: The decreases in liver glycogen are of greater magnitude than in the group receiving the smaller dose. The interpretation of the data on the muscles presents difficulties, on account of the variation in the controls. The $1\frac{1}{2}$ hours group showed an average

decrease twice as great as that of the controls, but the absolute difference is only 8 per cent of the initial value. The 3 hour group shows a greater decrease—12 per cent of the initial value. Although the decrease is slight, I feel that it is real, as it represents an average rather than individual results.

DISCUSSION. Although Simpson and Macleod (10) demonstrated in 1927 that the breakdown of muscle glycogen as a result of giving an animal a lethal blow on the head takes place with lightning rapidity, this method has remained a favored one for obtaining a numerical value supposed to represent the ante-mortem glycogen content of the muscle. As a result

TABLE 1
Liver and muscle glycogen in cats fasted 24 hours
Second samples taken 1½ hours (a) or 3 hours (b) after first

EXPERIMENT NUMBER	LIVER GLYCOGEN		MUSCLE GLYCOGEN	
	First	Second	First	Second
(a) Interval between samples, 1½ hours				
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
1	2.41	2.20	0.80	0.77
2	3.20	3.30	1.08	1.00
3	1.28	1.15	0.92	0.91
Average.....	2.30	2.24	0.93	0.89
(b) Interval between samples, 3 hours				
4	2.99	2.18	0.67	0.83
5	4.16	3.54	0.84	0.92
6	3.73	2.46	0.84	0.76
7	2.70	2.08	1.45	1.22
Average.....	3.40	2.57	0.95	0.93

we find in the current literature "normal" values ranging from a mere trace up to 1 per cent or more. It is not logical to expect that such a violent procedure can give results of any significance. Even though consistent values are obtained, they cannot be considered correct. Cori (3) has demonstrated that consistent values can be obtained on rats bled to death, but that they are about half as great as on muscles excised from anesthetized animals.

It would appear that this work of Simpson and Macleod has escaped the attention of many investigators, for there have been several papers published in 1929 and 1930 in which glycogen has been determined on muscles removed from animals killed by a blow on the head. Bischoff and Long

(1), Corkill (4), Eadie (7) and Sahyun and Luck (9) are among those who have used this method.

This is not intended as a brief for the use of the freezing method of Davenport and Davenport to the exclusion of other procedures. Cori (3) and Zimmerman (11) have shown by their results that it is not necessary on rats, in which the weight of the entire gastrocnemius is about one gram. Simpson and Macleod have a satisfactory method for use on larger animals: excision of the muscle from the anesthetized or decerebrate animal followed by immediate freezing in liquid air. I believe, however, that the use of carbon dioxide snow and a volatile liquid is more convenient and more generally useful than any other method.

TABLE 2
Liver and muscle glycogen in cats after epinephrine
Dose 0.1 mgm. per kilo

EXPERIMENT NUMBER	LIVER GLYCOGEN		MUSCLE GLYCOGEN	
	Before injection	1½ hours after injection	Before injection	1½ hours after injection
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
8	3.45	3.04	1.15	1.23
9	4.47	3.27	0.90	0.86
10	4.54	3.70	1.00	0.96
Average.....	4.15	3.34	1.02	1.02
		3 hours after injection		3 hours after injection
11	6.15	4.37	1.43	1.33
12	2.06	1.07	0.80	0.77
13	1.38	0.73	0.79	0.78
14	4.37	2.86	0.90	0.95
Average.....	3.49	2.26	0.98	0.95

The question of the possible effect of anesthesia on the glycogen content of the tissues is still before us. Hinsey and Davenport (8) and Simpson and Macleod have shown that there is no disappearance of muscle glycogen in decerebrate animals, and the former have shown that there is no disappearance under ether or amytal in three hours. This is confirmed by the data above. Liver glycogen does definitely diminish under amytal; the question is an open one whether it disappears more rapidly than in unanesthetized animals. In order to settle the question it will be necessary to perform experiments on animals in which chordotomy or decerebration has been done and time allowed for complete recovery from the anesthetic.

No evidence was found for the synthesis of glycogen under the influence

of epinephrine in the amyralized cat. As no data are available for the rabbit, dog, etc., it cannot be stated whether the behavior of the cat is exceptional or that of the rat is peculiar. In the light of present information, the two possibilities are equally plausible.

Eadie's earlier paper (6) reports that $1\frac{1}{2}$ hours after the administration of 1.0 mgm. of epinephrine per kilo, the liver glycogen is reduced practically to zero. The results above (table 3) show a marked decrease, but the amount of glycogen remaining is, on the average, ten times as great as in Eadie's experiments (0.78 per cent as compared with 0.08 per cent). Two possible explanations of this discrepancy present themselves. Eadie

TABLE 3
Liver and muscle glycogen in cats after epinephrine
Dose 1.0 mgm. per kilo

EXPERIMENT NUMBER	LIVER GLYCOGEN		MUSCLE GLYCOGEN	
	Before injection	$1\frac{1}{2}$ hours after injection	Before injection	$1\frac{1}{2}$ hours after injection
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
15	3.42	1.50	1.02	0.92
16	3.13	0.36	1.15	0.95
17	3.77	1.14	0.88	1.00
18	3.19	0.29	0.98	0.86
Average.....	3.38	0.78	1.01	0.93
		3 hours after injection		3 hours after injection
19	3.48	0.39	1.24	1.03
20	3.67	0.15	1.00	0.90
21	1.39	0.59	0.74	0.76
22	3.42	0.21	1.10	0.91
Average.....	2.99	0.35	1.02	0.90

waited three hours after the administration of the amyral before taking the first sample, while in this work the first sample was taken as soon as anesthesia was complete, generally $\frac{1}{2}$ to 1 hour after the amyral administration. This explanation is rendered more plausible by a comparison of the two control series. Eadie found that $\frac{1}{2}$ to $\frac{1}{3}$ of the liver glycogen of his controls disappeared in $1\frac{1}{2}$ hours, while in this series there was no loss in that time, but a disappearance of $\frac{1}{4}$ the total quantity was noted at the end of three hours. The second possibility is that glycogenolysis was arrested more rapidly by freezing 1 gram of liver than by boiling a 10 gram portion in water.

In view of the rather wide variations in glycogen content of the two

gastrocnemii of a control animal (table 1) it does not seem advisable to match a single control animal against a single injected one, as Eadie has done.

SUMMARY AND CONCLUSIONS

1. The glycogen content of the muscles of cats fasted 24 hours was found to range from 0.7 per cent to 1.4 per cent.

2. Under anesthesia induced by barbituric acid compounds the glycogen content of the liver was not appreciably affected in $1\frac{1}{2}$ hours, but was reduced $\frac{1}{4}$ in 3 hours.

3. Large doses of epinephrine caused a marked decrease in the liver glycogen of cats and a slight but definite decrease in the muscle glycogen.

4. Small doses of epinephrine caused a definite decrease in the liver glycogen of cats but had no demonstrable effect on that of the muscles.

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THE INFLUENCE OF VARYING AMOUNTS OF CARBOHYDRATE, FAT, PROTEIN AND WATER ON THE WEIGHT LOSS OF HOGS IN UNDER-NUTRITION¹

ROBERT W. KEETON, HELEN MacKENZIE, SELMA OLSON AND
LOUISE PICKENS

From the Department of Medicine, University of Illinois, College of Medicine

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Increase in weight is due to a discrepancy between caloric intake and expenditure. The cause of this discrepancy is still under discussion. In the earliest literature on obesity, fat individuals were divided into two classes; a, those due to exogenous, and b, those due to endogenous causes. It is implied that in endogenous obesity, a glandular dyscrasia exists, which changes the quantitative relationship existing in normal individuals and in those with exogenous obesity. Grafe (1921) defends this view but he does not define the difference between the two groups. Newburgh (1930) is emphatic in stating that this distinction between endogenous and exogenous obesity is a misconception.

An obese individual may live for a period of time on a diet distinctly below his caloric requirements without weight loss. This maintenance of weight is ascribed to a retention of water (Newburgh, 1930; Grafe, 1921; Lauter, 1926). To establish significant uniform weight loss all workers are agreed that under-nutrition must be established, but other requirements have been introduced into the diet upon which there is no general agreement. Some have suggested that the carbohydrate intake should be rigidly restricted and fats correspondingly increased. Others have advocated various levels of protein and still others consider the restriction of fluids and salts important.

Whether there are factors other than caloric deficiency which influence the magnitude of weight loss or the character of the curve during this loss seems to us worthy of study. Preliminary experiments were attempted with human subjects, but it was impossible to submit them to rigid experimental conditions necessary to answer these questions. So the hog was selected as a suitable subject. He was established in undernutrition and the influence of accessory factors on his weight curve were followed.

Effects of rations containing varying amounts of carbohydrates and fats. Ebsstein (1887) and Pfeiffer (1895) emphasized the importance of reducing

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the carbohydrates of the diet to a low point and raising the fats correspondingly. They maintain that such a diet gave better clinical results than could be obtained by Banting's diet (Howard, 1921). Weiler (1923) treated, with good results, a number of cases of obesity with a so called carbohydrate free diet. However his diets were not quantitative and were low in carbohydrate rather than free of carbohydrate. Hagerdorn (1927) from a study of respiratory quotients considers that obese persons convert carbohydrate into fat more rapidly than normal or thin individuals. Gordon and Stanley (1928) influenced by this work, have reported experiments in which the restriction of carbohydrate seemed to facilitate weight loss. Bischoff and Voit (1906) and Benedict and Milner (1907) have shown that a diet rich in carbohydrate confers on the body a certain ability to hold water. Newburgh (1929) states that a reducing diet rich in carbohydrate facilitates a uniform weight loss and tends to eliminate the periods of water retention. On the other hand a severe restriction in carbohydrates in the human subject leads to ketosis which is known to have a dehydrating effect (Gamble, 1923; McQuarrie, 1929; Fay, 1930).

In the first experiments, our hogs were fed rations of the same protein and caloric value, but the amounts of carbohydrates and fats were varied. When one animal was on a high carbohydrate ration, the other was on a high fat ration. The experiment was afterwards repeated with a change in the rations. In this way individual variations and environmental conditions were controlled.

PLAN OF THE EXPERIMENTS. Two male hogs, 10 months of age, of the same litter, of the same approximate size and raised on skimmed milk and corn were secured for the experiment. For our purpose it was considered important to have mature animals, and yet it is a little difficult to define maturity in the hog for he continues to grow in the second and third years if sufficient food be given. However in animals weighing 125 to 150 kilos in one year it was considered that the growth factor could be neglected. The hogs were placed in metabolism cages such as have been described by McCollum and Steenbock (1912).

For feeding and weighing they were transferred each morning to a special feeding box which was placed on scales. The metabolism cages were then cleaned and the animals returned to them after feeding. Water was supplied by a galvanized iron tank attached to the outside of the cage. This tank (Novelty Iron Works) was connected by a ball valve to a drinking trough on the inside so that a constant level was maintained. Any water spilled over the side of the trough was caught in the urine jar.

The analytical results were obtained on specimens of urine and feces collected over a five day period. The first collection period was usually begun after the animal had been on a given diet from five to ten days. The second period covered the last five days of the experimental period.

Some difficulty was experienced in getting quantitative collections of the

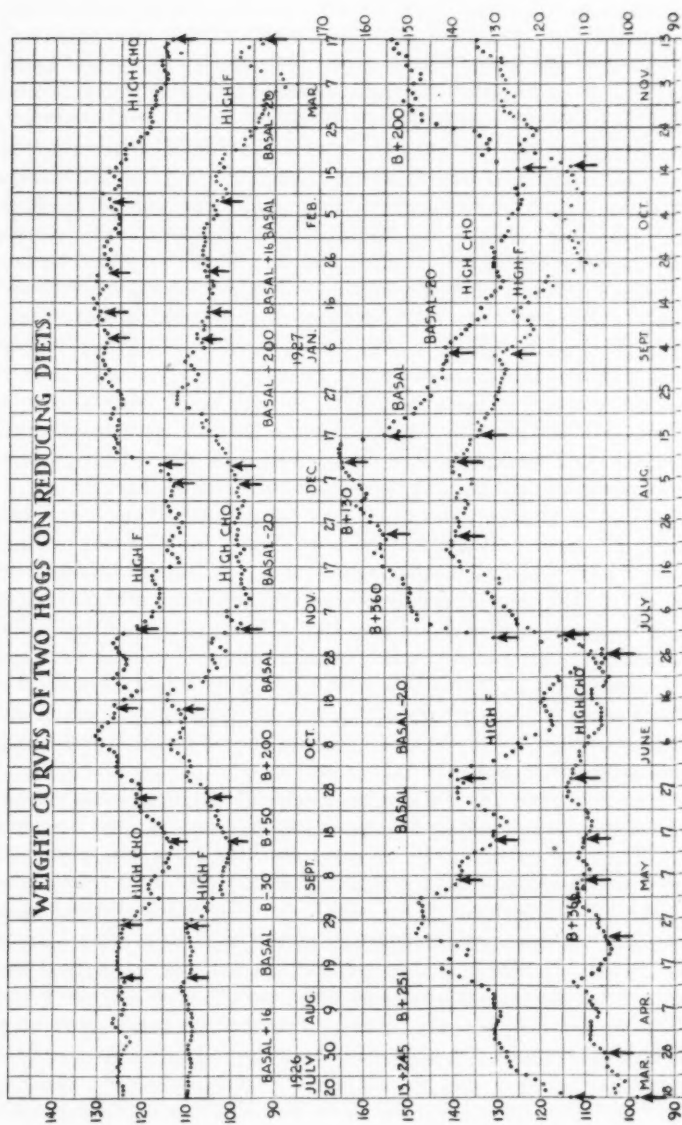


Chart 1

feces. The cages were watched and feces removed shortly after passage. When the animals were on a sub-maintenance ration there were times when part of the feces was eaten. This source of error was minimized by the fact that the animals usually defecated following the morning feeding. The hog's bladder has a capacity of two liters or more. It was out of the question to catheterize the animals. Hence the collection periods were begun and closed immediately after the animal had emptied his bladder.

The animals were fed a maintenance ration with a protein value of 0.94 gram per kilo body weight, for approximately fourteen days. During this time the weight became stabilized and one collection period was obtained. The experimental period with a sub-maintenance ration followed. The ration in the case of one hog contained as much carbohydrate ("high carbohydrate") and in the case of the other as much fat ("high fat") as was consistent with the 0.94 gram of protein per kilo of body weight, and the caloric level selected. This experimental period lasted from twenty to forty days, and between the experiments the animals were fed liberally until recovery had occurred. The duration of time allowed for recovery and the quantity of food fed can be seen by reference to chart 1.

ANALYTICAL METHODS. The urine was tested for acetone by Legal's nitro-prusside reaction (Hawk and Bergeim, 1926); for albumen by nitric acid and by heat coagulation after acidification. The total nitrogen was determined by the macro-Kjeldahl method; the ammonia nitrogen by the permittit micro-method of Folin and Bell (1917); creatinine by Folin's micro chemical method (1914) and titratable acidity by Folin's method.

The total nitrogen of the feces was determined by Kjeldahl method on moist feces using potassium sulphate as the catalytic agent. An aliquot portion of each days collection was stored in the refrigerator and a composite sample made up at the end of the period.

Maintenance rations. Voit's figures (1901) for the hogs basal requirements (19.1 calories per kilo) were adopted as the basis for computation of rations. Since the animals were in cages and had little opportunity for activity it was considered that a ration of 16 per cent above their basal requirement (22.1 calories per kilo) should be adequate for weight maintenance. In a preliminary experimental period of 27 days from June 20, 1926 to August 17, 1926 it was found that the animals maintained their weight on such a ration. In a subsequent period of twelve days on a basal ration this weight remained unchanged. Hence these ranges may be regarded as maintenance for the animals under the conditions of the experiment.

Basal plus 16 per cent ration. The basal plus 16 per cent ration is shown in table 1. The analyses are taken from Henry and Morrison (1927).

The caloric value of carbohydrate and protein was reckoned as four, and of fat as nine. Each gram then of the above ration gave 2.901 calories

and 7.586 grams were used to furnish 22 calories per kilo. Canned tomatoes (1.3 gram per kilo) were added, making the ration consist of carbohydrate 3.97 grams, protein 0.94 gram, fat 0.29 gram per kilo, total calories 22.25. This represented a basal plus 16 per cent.

Basal ration. By reducing the percentage of the corn in the ration to 20 per cent, by increasing the linseed oil meal to 18 per cent and adding 1.3 gram of canned tomatoes per kilo body weight a ration was obtained with these figures: Carbohydrate 3.27 grams, protein 0.94 gram, fat 0.25 gram and calories 19.1 per kilo body weight.

Experimental rations. In the experimental period beginning August 14, 1926 the rations were cut to 30 per cent below the basal requirements. In the period beginning December 2, 1926 the ration was cut to basal minus 20 per cent. In order to supply vitamins and the proper protein

TABLE I
Composition of basal plus 16 per cent ration

FOODS	WEIGHT	COH	PROTEIN	FAT	WATER
	<i>per cent</i>	<i>grams</i>	<i>grams</i>	<i>grams</i>	<i>grams</i>
Corn.....	28.0	18.98	2.10	1.288	2.94
Oats.....	25.0	13.03	24.25	0.950	2.30
Wheat middlings.....	30.0	13.86	4.02	1.290	3.15
Linseed oil meal.....	10.0	3.79	3.17	0.280	0.96
Alfalfa.....	5.0	1.95	0.53	0.045	0.43
Sodium chloride.....	2.0				
Total.....	100.0	51.61	12.245	3.853	9.78

requirement a basic nucleus consisting of irradiated alfalfa, canned tomatoes, tankage and cod liver oil was used. To this nucleus was then added either oil or starch to bring the calories to the required level. Tables 2 and 3 show the basic nucleus and the value of the rations derived by the addition of starch and oil.

In the further experiments in which under-nutrition was established at a level of 20 per cent below the basal requirements, the "basal nucleus" was omitted from the ration so that the high fat ration contained no preformed carbohydrate. While these latter rations were lacking in vitamins it was thought that, since the average duration of the experimental periods was only thirty days this was not a serious objection.

EXPERIMENTAL RESULTS. *Basal rations.* The analytical results of the collection periods obtained while the animals were on basal rations are summarized in table 5. It will be noted that the animals continued to store nitrogen in all experiments except two. In the experiment of May 15, 1927 the fecal nitrogen shows a very small value. The collections

doubtless had not been complete due to the eating of feces by the hogs. If this value were increased to 3.0 grams, then animal I would be in approximate equilibrium and animal II would be storing about 2.3 grams per day. In experiment August 15, 1927 both animals are in equilibrium. This difference in the behavior of the animals is doubtless due to the fact that their rations had been rapidly reduced from values of basal plus 300 per cent to basal; over a short period of time. Sufficient time had not been allowed for stabilization at each lower level. It seems quite interesting to

TABLE 2
Basic nucleus for sub-maintenance rations

FOODS	WEIGHT	COH	PROTEIN	FAT
	gram per kgm.	gram per kgm.	gram per kgm.	gram per kgm.
Irradiated alfalfa.....	0.379	0.148	0.040	0.003
Tomatoes.....	1.300	0.052	0.0156	0.0026
Tankage.....	1.515		0.8892	0.1909
Cod liver oil.....	0.025			0.025
Total.....		0.200	0.9448	0.2215

TABLE 3
Sub-maintenance rations, earlier experiments

TYPES OF RATIONS	FOOD		COH	PRO- TEIN	FAT	CALO- RIES
		Amount				
		grams per kgm.	grams per kgm.	gram per kgm.	gram per kgm.	grams per kgm.
Basal—30% high fat.....	Basal nucleus oil	0.7555	0.200	0.9448	0.977	13.372
Basal—30% high COH...	Basal nucleus starch	1.6996	1.8996	0.9448	0.2215	13.372
Basal—20% high fat.....	Basal nucleus oil	0.9701	0.200	0.9448	1.1916	15.300
Basal—20% high COH...	Basal nucleus starch	2.1827	2.3827	0.9448	0.2215	15.300

note that at this lowest weight maintenance level the animals continue to store nitrogen. The values for ammonia nitrogen, creatinine nitrogen and titratable acidity are within the expected limits.

Basal minus 30 per cent rations. In the first experiment the ration was cut to 30 per cent below the basal level. The results are summarized in table 6. It will be noted that both animals developed a negative nitrogen balance and an increase in creatinine. No increase occurred in the titratable acidity. In chart 1 the weight variations are plotted. Each point

represents an average weight of three days. In this way minor fluctuation are smoothed out. The weight curves of the two animals behaved simi-

TABLE 4
*Sub-maintenance rations, later experiments**

TYPES OF RATIONS	FOOD		COH	PROTEIN	FAT	CALORIES
		Amount				
		grams per kgm.	grams per kgm.	gram per kgm.	gram per kgm.	grams per kgm.
Basal—20% high fat.....	Tankage	1.687		0.9447	0.1215	
	Oil	1.1565			1.1565	
Total.....				0.9447	1.278	15 28
Basal—20% high COH....	Tankage	1.687	2.602	0.9447	0.1215	
	Starch†	2.602				
Total.....			2.602	0.9447	0.1215	15 28

* Experiment dates, 2-9-27; 5-29-27; 9-4-27.

† The starch was made into a paste with water and then tankage was added.

TABLE 5
Nitrogen excretion on basal rations

ANIMAL	DATE AND DURATION OF EXPERIMENT	AMMONIA N ₂	CREATININE N ₂	TITRATABLE ACIDITY	TOTAL NITROGEN			BALANCE
					Intake	Output		
						Urine	Feces	
		grams	grams	cc.				
I	8-14-26	1.003	2.021	509	18.588	9.055	2.850	+6.683
II	14 days	0.725	2.628	259	16.472	6.863	3.540	+6.069
I	10-15-26	0.830	1.643	574	18.588	9.590	2.581	+6.417
II	18 days	0.878	2.734	180	16.472	7.189	3.907	+5.376
I	1-23-27	1.056	3.252	526	18.588	8.594	2.478	+7.516
II	17 days	1.470	2.764	249	16.472	9.094	4.458	+2.920
I	5-15-27				18.587	15.700	0.443	+2.444
II	15 days				16.472	10.537	0.618	+5.317
I	8-15-27				20.010	16.024	3.300	+0.67
II	19 days				16.774	13.555	3.100	+0.11
I	12-28-27				24.030	12.310	3.400	+8.30
II	11 days				20.690	8.960	3.300	+8.43

larly. Since the animals developed a negative nitrogen balance it was thought that the cut had been too severe and that possibly some variations

in weight might be shown if the nitrogen balance was maintained. So in the remaining experiments the rations were cut to a basal minus 20 per cent level.

TABLE 6
Nitrogen excretion on basal minus 30 per cent rations

TYPE OF RATION	DATE AND DURATION OF EXPERIMENT	HOG NUMBER	AMMONIA N ₃	CREATININE N ₂	TITRATABLE ACIDITY	TOTAL NITROGEN			BALANCE
						Intake	Output		
							Urine	Feces	
High COH	8-28-26 19 days	I	1.67	3.75	292.0	18.594	19.371	5.00	-5.7
High fat	8-28-26 19 days	II	1.305	2.50	215.0	16.477	15.956	4.45	-3.93

TABLE 7
Nitrogen excretion on basal minus 20 per cent rations

TYPE OF RATIONS	DATE AND DURATION OF EXPERIMENT	HOG NUMBER	AMMONIA N ₂	CREATININE N ₂	TITRATABLE ACIDITY	TOTAL NITROGEN			BALANCE
						In-take	Output		
							Urine	Feces	
High COH	11-2-26 34 days	II	1.18	2.84	292.0	16.48	13.03	0.54	+2.91
	2-9-27 36 days	I	1.07	4.04	200.0	18.59	13.29	4.56	+0.74
	5-29-27 28 days	II	2.88	2.30	222.9	16.77	15.47	2.19	-0.89
			2.65	2.54	391.1	16.77	15.14	2.25	-0.62
	9-4-27 41 days	I	2.75	1.81	84.0	20.01	20.67	3.55	-4.20
3.02			1.66	59.0	20.01	15.70	5.05	-0.74	
High fat	11-2-26 34 days	I	1.65	3.92	580.0	18.59	17.62	0.45	+0.52
	2-9-27 36 days	II	0.96	3.66	130.0	16.48	12.99	2.20	+1.29
	5-9-27 28 days	I	2.63	3.19	95.5	20.01	17.26	1.51	+1.24
			2.43	3.16	333.7	20.01	18.71	2.03	-0.73
	9-4-27 41 days	II	2.86	1.69	37.0	17.76	17.28	3.2	-2.72
6.82			2.09	0.0	17.76	16.85	2.6	-1.69	

Basal minus 20 per cent. By reference to chart 1 and table 7 it will be noted that each animal was run through two experimental periods on the

high fat rations and through two periods on the high carbohydrate rations. It will be further noted that when hog I was on the high fat ration hog II was on the high carbohydrate ration. In this way the weather and environmental factors were controlled.

From table 7 it will be noted that the animals remained very close to nitrogen equilibrium throughout the experimental periods and that the high carbohydrate ration was not more effective in holding the animals in equilibrium than the high fat ration.

The urines were repeatedly tested for acetone and in no case was a positive test obtained. The ammonia values were somewhat higher than those found on the basal rations. In only one case (hog II, expt. Sept. 4, 1927) was the value significantly increased. It will be noted that in this period the urine was alkaline. The urine of both animals on both rations tended to become more alkaline in the later experiments which may account for some of the fluctuations in the ammonia and creatinine nitrogen.

Plasma carbon dioxide estimation of blood obtained from the tail, at the close of an experimental period showed normal values after each ration. We may conclude that an acidosis did not develop on either ration.

At this point it is interesting to examine more critically the high fat ration. It will be noted that it contained 0.94 gram of protein per kilo. Of this 58 per cent or 0.545 gram would be converted into glucose (Wood-yatt, 1921; Lusk, 1928). It may be assumed that the animals were utilizing calories equivalent to their basal requirements (19.1 calories per kilo). If the fatty acids values derived from the protein (46 per cent) and from the fat (90 per cent) be computed, they will be 1.966 gram per kilo. If this is divided by the glucose of the diet then the ratio of fatty acids to glucose in the metabolizing mixture will be 3.6. This value is well over the threshold of ketogenesis as determined for a normal man, and is in agreement with Mason's (1927) findings in the human obese subject.

In chart 1 it will be seen that the weight curves run a strikingly parallel course. So that it is concluded that within the limits of these rations the presence of large or small quantities of carbohydrate or the absence of all preformed carbohydrate exerts no specific effect on weight loss. In other words, the weight loss was a function of the caloric intake and was not influenced by the source of the calories. The daily weight fluctuations also were not influenced by the varying amounts of carbohydrate in the rations.

Effects of rations containing varying amounts of protein. The earlier writers on obesity, Banting, Ebstein, Oertel and Van Noorden (Howard, 1921) have stressed the importance of feeding patients placed on reducing diets, relatively large quantities of protein. It was considered that such a quantity of protein would protect the body against a negative nitrogen balance, and would promote weight loss through its specific dynamic action. Quite a controversy (Hirschfeld, 1894; Dapper, 1898) arose as to

whether patients on such diets remained in nitrogen equilibrium, but the evidence favored the view that an equilibrium was maintained. In a group of normal subjects, Benedict, Miles, Roth and Smith (1919) observed a negative nitrogen balance when the calories were cut 50 to 56 per cent.

Mason (1924) considers it unnecessary to maintain patients with exogenous obesity in nitrogen equilibrium as serious nitrogen losses do not occur. Later (1927) he studied patients on diets low both in calories and protein and pointed out the strong tendency to conserve nitrogen equilibrium. He concludes that patients on such diets who lost weight most rapidly also lost more body nitrogen per day. The deleterious effect on man of diets

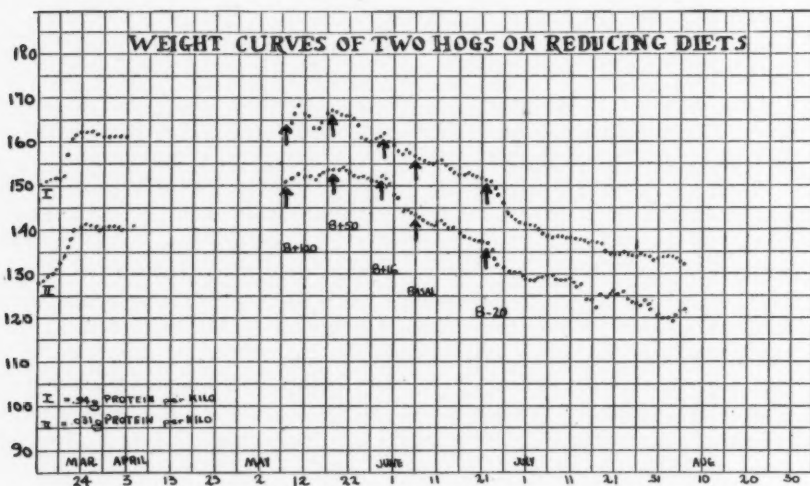


Chart 2

low in calories and protein when continued over a period of time has been summarized by Lusk (1921).

For the above reasons clinical writers (David, 1925; Schellong, 1928; Spencer, 1928; Strang, 1930) continue to emphasize the importance of feeding relatively large quantities of protein during the reducing process. However we have been unable to find any experiments in which the question was studied as to whether a variation of the protein content of the diet or the development of a negative nitrogen balance has any effect on the rate of weight loss or the character of the curve during reduction.

Experimental details. Reference to chart 2 will show that the animals had been on fattening rations during the months of March, April and May,

1928. During the month of June the rations were reduced until the basal ration was reached on June 6th and the animals were kept on this for 17

TABLE 8
Composition of rations

ANIMAL	TYPE OF RATION	FOODS USED	COMPOSITION PER KILOGRAM			
			COH	Protein	Fat	Calories
I; II	Basal	Corn Oats Wheat middlings Alfalfa Linseed oil	3.27	0.94	0.25	19.1
I	Basal—20%	Tomatoes Starch Casein	2.43	0.94	0.2	15.28
	Basal protein	Oil				
II	Basal—20%	Tomatoes Starch	2.43 ₄	0.03	0.6	15.24
	Starvation protein	Oil				

TABLE 9
Nitrogen excretion on basal and sub-maintenance rations

TYPE OF RATION	DATE AND DURATION OF EXPERIMENT	HOG NUMBER	URINE ANALYSIS				FECAL N ₂	INTAKE N ₂	BALANCE
			Ammonia N ₂	Creatinine N ₂	Titratable acidity	Total N ₂			
Basal	6-11-28 17 days	I	12.02	3.19	Alkaline	13.67	4.67	24.06	+5.72
		II	7.42	3.01	232	19.08	5.68	21.05	-3.71
Basal—20%	7-5-28 47 days	I	15.08	3.51	Alkaline	18.61	0.6	23.08	+3.87
Basal protein	7-29-28 47 days	I		3.03	Alkaline	26.91	0.2	23.08	-4.03
Basal—20%	7-5-29 47 days	II	5.88	5.13	206	9.5	0.23	0.69	-9.04
Starvation protein	7-29-28 47 days	II		3.29	100	8.11	0.11	0.69	-7.53

days. The composition of the basal and experimental rations is shown in table 8. On June 22, 1928 the basal requirements of the animals were

recalculated on the basis of their weights at this time, and the experimental rations were instituted. The hogs were continued on these rations for 47 days. Analytical periods were started on the basal ration June 11, 1928 and on the sub-maintenance rations on July 5, 1928 and July 29, 1928 respectively.

RESULTS. By reference to table 9 it will be noted that during the basal period one of the animals (I) was storing nitrogen and the other (II) was in slightly negative balance. It will be seen from chart 2 that the weight of the animals were not as stationary during this period as one might desire. This slight negative balance and further moderate decline in weight suggest that the reduction in the rations had been too rapid so that the stimulating effects of the "luxus consumption" present during the fattening had not been entirely lost. For the purposes of the experiment this is unimportant since the weight curves of both animals follow each other closely. Indeed it was rather fortunate that the animal which was showing a negative nitrogen balance in the basal period should have received the ration containing a starvation protein. In this way the full effect on weight loss of a definite negative balance can be best seen.

During the experimental period, both animals lost weight at equal rates. The curves are strikingly similar. Animal I, which was fed the ration containing the same protein as the basal ration, developed a slight negative balance toward the close of the period. Animal II which was fed the starvation protein developed a negative balance of 9.04 grams at the beginning of the period and 7.53 grams at the close, an average of 8.3 grams. This value is rather lower than one might expect. It indicates the frugality of the body in using its protein when other sources of energy are available.

In a man of 70 kilos body weight, the wear and tear or minimal quota of protein corresponds to 2.5 to 3 grams nitrogen per day (Lusk, 1928). If these figures are applied to the hog the wear and tear quota on hog II whose weight during the basal period was 139.5 kgm. should be 5 to 6 grams nitrogen per day. Since these rations had appreciable quantities of carbohydrate in them, and since it has been previously shown that the hog does not develop ketosis when fed rations with a fatty acid to glucose ratio of 3.6 to 1, it is safe to conclude that none of the urinary nitrogen in the case of hog II represents protein requisitioned for its anti-ketogenic effect. The remaining 2 or 3 grams of nitrogen per day may be attributed to cellular nitrogen liberated by the reducing process. This quantity of nitrogen is not sufficient to warrant the assumption that the 17 kilo of body weight lost was muscle. Indeed the nitrogen would be the equivalent of only $\frac{1}{4}$ to $\frac{1}{3}$ of that amount of muscle. It is therefore highly probable that the weight lost represented chiefly fat and its intimately associated fluids and protein.

The effects of rations containing varying amounts of water. One of the early dietetic attempts at the treatment of disease was that of Schroth which was given something of a scientific standing by Cantani (Ebbard, 1910). In this diet the patients eliminated all the ordinary foods and drinks and were fed exclusively on stale, thoroughly dried rolls. On the third or fourth day of this diet a bottle of light wine was served. This treatment was used in a variety of diseases including obesity. It caused the patients considerable suffering, usually induced a negative nitrogen balance and often a fever.

Oertel (1891) noted that many obese patients have a certain degree of circulatory embarrassment and that excellent results could be obtained in such cases by restricting the fluid intake. His method of treatment which included a restriction of the calories also, was widely used and from the results obtained the clinicians of his day concluded that limitation of fluids exerted a specific effect on fat catabolism.

Von Noorden (1907) after a critical review of the subject, considered that although the restriction of fluids may be valuable in many clinical situations it is not effective in treating cases of obesity in otherwise healthy persons. Hirschfeld (1895) and Salomon (1905) reached similar conclusions.

Denning (1899) subjected patients to diets adequate in calories but deficient in fluids. Such patients became restless, slept poorly, and were unable to eat all of the food offered them. They lost weight rapidly. However no significance could be attached to weight lost under these conditions of starvation and increased energy requirements. Spiegler (1901) found that dogs could maintain their urinary output in the face of very severe restriction of water. The weight lost was appreciable in spite of the maintenance of their caloric intake. Straub's results (1899) agree with those of Spiegler. Pernice and Scagliosi (1895) deprived dogs of water entirely and fed them stale bread. Since their animals failed to eat all of their food and died in the course of the experiment the weight variations are not significant.

The idea that weight reduction in cases of pure obesity may be facilitated by water restriction and the use of diuretics has persisted to the present day (Eppinger and Kisch, 1925; Umber, 1925; David, 1925; and Grafe, 1929). It is not clear from these studies whether the authors expected to induce simply a more uniform weight loss or whether they believed that the use of diuretics and water restriction would establish a greater weight loss.

Experimental details. The two hogs, were established in under-nutrition. To one water was given ad libitum and to the other the quantity was restricted below his daily requirements. This restriction however was not so great as to interfere with the eating of all of the ration or with the secretion of urine. This animal showed none of the restlessness described in previous experiments (Denning, 1899). On the contrary he was rather

apathetic and slept more than his companion, to whom water was given freely.

The preceding experimental period in which the animals were on a sub-maintenance ration was closed October 15, 1927. They were placed at once on the standard ration which was supplemented with canned tomatoes and cod liver oil. During this recovery period the calories in the ration were raised to basal plus 200 per cent or 57.3 calories per kilo. The animals gained in weight from 112 and 125 kilos respectively up to 137 and 160 kilos by December 14, 1927. Their rations were then reduced to 28

TABLE 10
Composition of rations

TYPE AND ANIMAL	FOODS	COMPOSITION PER KILOGRAM					WATER
		Weight	COH	Protein	Fat	Calories	
Standard basal Hogs I; II	Corn	1.309	3.274	0.9445	0.2477	19.1	cc. Ad libi- tum
	Oats	1.636					
	Wheat midlings	1.964					
	Linseed meal	1.178					
	Alfalfa	0.327					
	Tomatoes	1.300					
	Sodium chloride	0.131					
Basal—20% Hog I	Alfalfa	0.569	2.092	0.9492	0.3954	15.72	Ad libi- tum
	Tankage	1.515					
	Cod liver oil	0.200					
	Starch	1.870					
	Sodium chloride	0.110					
Basal—20% Hog II	Alfalfa	0.569	2.092	0.9492	0.3954	15.72	1,700
	Tankage	1.515					
	Cod liver oil	0.200					
	Starch	1.870					
	Sodium chloride	0.120					

calories per kilo on December 14, 1927 and to 22.15 calories per kilo on December 17. On December 28, they were placed on their basal requirements of 19.1 calories per kilo, and remained on this until January 20, 1928 when the experimental ration (basal minus 20 per cent) was instituted. Three months had elapsed since their last experimental period and during this time there could be no question as to the adequacy of their rations. The experiment lasted 18 days and was terminated February 8, 1928, the first day on which hog II whose water was restricted was unable to eat his complete ration. Following the return of water he recovered in a short time. Between February 8 and February 25 (17 days) both the animals

were kept on basal minus 20 per cent rations with liberal allowances of water. On February 25 they were returned to their basal requirements as estimated on December 28, which because of their weight loss was the equivalent of 22 calories per kilo. Therefore this ration furnished calories in excess of their actual requirements thus affording an opportunity for the establishment of a gain in weight. On March 15, the rations were increased to basal plus 200 per cent (57.3 calories per kilo) and the experiment was terminated.

The composition and quantitative values of the basal and the experimental rations (basal minus 20 per cent) are shown in table 10. It will be noted that in the case of hog II the intake of fluids was limited to 1700 cc. which was regarded as an amount below his requirements. If we assume that the hog eliminates heat in much the same manner as man, and that

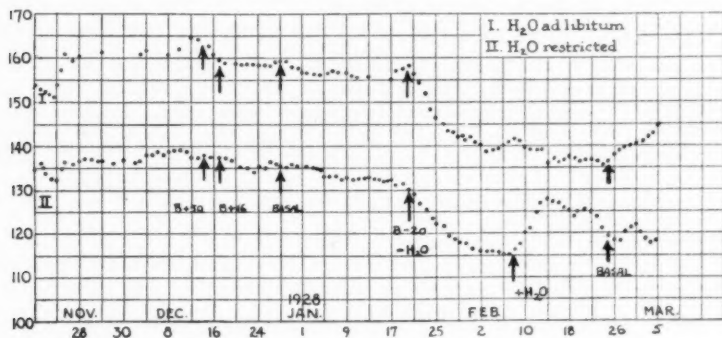


Chart 3

25 per cent of his calories will appear as heat of vaporization, then approximately 800 cc. will be required for this. Another 800 cc. would be required for the urine and 200 cc. for the feces.

The alfalfa and tankage contained 23.1 cc. of water. As a result of the oxidation of 286.6 grams of carbohydrate, 130.04 grams of protein and 54.17 grams of fat, 270.78 cc. of water would be developed. This gave a total of 293.88 cc. or 294 cc. for water content of the diet. The ration was then made up with 1406 cc. of water which made a total intake of 1700 cc. for the day.

RESULTS. By reference to chart 3 it will be noted that the rate of weight loss was approximately the same in the two animals. This is quite striking when it is realized that hog II was evidently short of fluids and presented definite evidences of this shortage by absence of saliva, a dry mouth and tongue, and finally by inability to swallow food. On February 28, he was allowed water ad libitum and immediately his intake rose to approxi-

mately 18,000 cc. per day. His weight increased correspondingly but it may be noted that after 14 days it had again dropped to almost the same level from which it had risen when the water intake was increased. It is probable that while the shortage of water was manifesting itself in the decrease or absence of some of the external glandular secretions (saliva and mucus the desiccation was not sufficient to alter appreciably the intracellular structure or activity.

The intake of water of hog I averaged 24,191 cc. per day during the nineteen experimental days. During the five days of the collection period the intake was only 19,992 cc. per day. It was found during periods of under-nutrition that the intake of water was often determined by the amount of water offered the animal. No attempt has been made to study

TABLE 11

Nitrogen excretion on basal and basal minus 20 per cent rations—varying amounts of water

TYPE OF RATION	DATE AND DURATION OF EXPERIMENT	HOG NUMBER	WATER		NITROGEN			
			Intake	Urine	Urine	Feces	Food	Balance
			cc.	cc.	gram	gram	gram	gram
Basal	12-28-27 23 days	I	23,191	17,560	12.31	3.42	24.03	+8.18
	12-28-27 23 days	II	20,792	17,460	8.96	3.3	20.6	+8.34
Basal-20%	1-20-28 19 days	I	19,992	14,300	22.82	4.62	24.03	-3.41
	1-20-28 19 days	II	1,700	892	15.27	5.5	20.6	-0.17

the cause for this variation in water intake. It was assumed that the animal drank this additional water because of hunger.

The nitrogen balance (table 11) during the basal periods was positive in both animals and during the experimental periods was quite comparable in the two animals.

SUMMARY

1. Approximately mature hogs confined in metabolism cages maintain their weight on basal requirements when such requirements are estimated according to Voit's figures of 19.1 calories per kilo of body weight.

2. On basal rations, hogs are in positive nitrogen balance and at times appear to be storing nitrogen.

3. In cage life on rations 30 per cent below basal requirements, including

0.94 gram of protein per kilo and wide variations in carbohydrate, they develop a negative nitrogen balance. Their loss in weight paralleled the caloric deficiency.

4. In cage life and on rations 20 per cent below their basal requirements including 0.94 gram protein per kilo body weight, they remain in nitrogen balance but lose weight.

5. All sub-maintenance rations, which were 20 per cent below basal requirements, contained 15.28 calories per kilo body weight. Three types of secondary variations were introduced:

a. *Variations in carbohydrate and fat:* To both animals 0.94 gram of protein per kilo body weight was fed. To one animal no carbohydrate was given and to the other as much carbohydrate, as was consistent with the calories selected. Fat furnished the remainder of the calories.

b. *Variations of protein:* To one animal a protein of 0.94 gram per kilo and to the other a protein of 0.03 gram per kilo was fed. Fat and carbohydrate furnished the remainder of the calories.

c. *Variations of water:* In this case the ration of both animals contained the same carbohydrate, fat and protein. To one animal water was given ad libitum. He took 24,191 cc. per day. To the other animal 1700 cc. per day was given.

Through all of these variations the hogs lost weight at the same rate. The loss was uniform without evidence of water retention, and parallel to the caloric deficiency.

6. On the high fat ration the fatty acid to glucose in the metabolizing mixture approximated a value of 3.6, which is higher than the threshold of ketogenesis in normal men. The animals showed no ketosis.

7. The weight loss of the hog fed 0.03 gram of protein per kilo can be ascribed to fat, and its intimately associated fluids and protein. There was no significant destruction of body protein as shown by the nitrogen excretion.

8. The hog with the limited intake of water (1700 cc.) after 19 days, showed evidence of desiccation, absence of saliva and a dry mouth. Evidence is presented that this desiccation was not sufficient to alter appreciably the intra-cellular structure or activity.

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THE INFLUENCE OF SPINAL IRRADIATION ON CUTANEOUS SENSATIONS¹

I. THE LOCALIZATION OF PAIN AND TOUCH SENSATIONS UNDER IRRADIATION

E. GELLHORN, H. GELLHORN AND J. TRAINOR

From the Department of Animal Biology of the University of Oregon, Eugene

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In a series of papers E. Gellhorn and his co-workers studied the influence of previous or simultaneous alteration of the retina with certain colors upon after images, retinal rivalry, field of vision, discrimination threshold and so on. The result was a more intimate knowledge not only of physiological processes of the retina but also of the cortex, since a characteristic inhibitory effect could be demonstrated if one eye was previously stimulated and the effect of this stimulation on certain phenomena as exhibited in the other retina was studied. The basic thought of the present and some following papers is rather similar. While in the former papers the influence of a cortical alteration upon the course of retinal sensations was studied, the present work deals with the effect of a spinal alteration upon cutaneous sensations. In previous papers, as well as in the present, the behavior of the sensations in question is regarded as furnishing an indication of the processes occurring in the central nervous system.

The importance of this problem is two-fold. First, the paper will give evidence of the fact that the grey matter of the medulla spinalis may influence in a very specific way sensory impulses conducted to the brain. Second, the facts to be presented are of greatest interest for a neuro-pathological point of view. The experimental results show clearly that the simple conception of hyper- and hypo-esthesia is unsatisfactory to describe fully the complexity of the phenomena when the central nervous system is involved. It is to be hoped, therefore, that the application of these physiological studies to pathological cases may lead to an improvement in diagnosis and a better understanding of the patho-physiological mechanisms in diseases of the spinal cord.

In 1917 Goldscheider showed in several papers that if a small portion of the skin is "pinched up," a large hyper-esthetic area is produced which extends chiefly in proximal direction. The area corresponds to the spinal segment stimulated and not to the distribution of the peripheral nerve.

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Therefore Goldscheider's method makes possible the examination of cutaneous sensations under the influence of spinal irradiation. This paper will describe the influence of spinal irradiation on the localization of touch and pain sensations.

METHOD. The distal third on the flexor side of the observer's forearm, as free as possible from hairs, was selected and a rubber stamped design of twenty-five squares, each 64 mm.², was printed thereon. This design served to locate the points with which we experimented. An identical design was stamped upon the experimenter's record sheet and on this the location of each selected point was marked. In each experimental sitting at least four points were selected.

Each point was stimulated with an esthesiometer (von Frey's) and the observer located, with a cork-tipped pointer which he held in the other hand, the point which he had felt as being stimulated. The point as localized by the observer was then marked on the record sheet and joined with a line to the point actually stimulated by the experimenter. These distances, the errors of localization, were afterwards measured in millimeters. In the case of touch the esthesiometer contained a horse-hair, and in the case of pain it contained a hair, with a thistle attached. The pressure exerted varied in the touch experiments from 0.95 gram to 1.1 gram and in the pain experiments from 2.3 grams to 2.5 grams.

After the localization of the points under normal conditions had been recorded, as described above, an irradiation condition was produced by placing slightly below the mapped area a clamp (described by Schriever) exerting a pressure of about 1200 grams over an area of 9.6 mm². It was left on approximately five minutes, or until it was certain that the irradiation condition had been produced. This may be determined by stimulating the area with the blunt end of the pointer and if the desired condition be present, the observer will report a peculiar numbness, which is all the more apparent if the other arm be stimulated in similar fashion and the comparison of the two sensations noted.

In some of the experiments the clamp was then removed and after a few seconds had been allowed for the sharp pain peculiar to the removal to disappear, the experiment was repeated. In other experiments the clamp was allowed to remain on while the localization experiments were carried out. It was imperative, however, that during the period of experimentation proper there be no pain at the area of the clamp.

In some of the experiments a third sitting under control conditions was carried out, it first being determined that the irradiation condition had disappeared.

Eight subjects were used in the experiments with pain points and four in the experiments with touch points. One complete experiment, consisting of at least one control and one irradiation experiment, was carried

out on the same day. Every precaution was taken that the conditions remain constant. The experimenters had had many practice trials with

TABLE 1

	CONTROL					IRRADIATION				
	Point A	Point B	Point C	Point D	Point E	Point A	Point B	Point C	Point D	Point E
Average.....	14.5*	14.5	12.5	12.0	13.5	4.5	7.0	4.0	4.5	4.75

* Localization error, in millimeters.

TABLE 2

Influence of spinal irradiation upon the localization of pain points
(Summary of all experiments on pain points)

SUBJECT	STIMULA- TIONS PER POINT	A CONTROL	B IRRADI- TION*	$\frac{B-A}{A} \times 100$
McC (male).....	4	19.15*	11.7	-39
	4	17.4	8.7	-50
B (male).....	6	8.2	6.5	-21
	6	11.0	8.7	-21
H (female).....	4	13.3	5.0	-62
M (female).....	6	10.1	7.8	-23
	6	10.7	8.0	-25
J (female).....	6	8.6	5.7	-34
S (female).....	8	6.9	5.2	-25
	8	8.0	6.4	-20
	8	7.9	6.2	-22
L (female).....	4	11.2	4.4	-61
	4	10.0	4.4	-56
G (female).....	8	9.1	4.7	-48
	8	7.9	3.8	-52
	8	8.5	5.9	-31
	8	8.6	5.0	-42
	8	7.6	6.0	-21

* The figures given are the averages of the means of the localization errors, expressed in millimeters.

the esthesiometers so that a high degree of motor skill had been attained in applying the esthesiometer. The results of one experimenter were checked by another without knowledge of the results of the former. The

observers always started to localize with the free hand in the same position, and they were at all times blindfolded. They were given preliminary practice in the localization of the stimulations. Experiments were not undertaken when either the observer or the experimenter expressed fatigue.

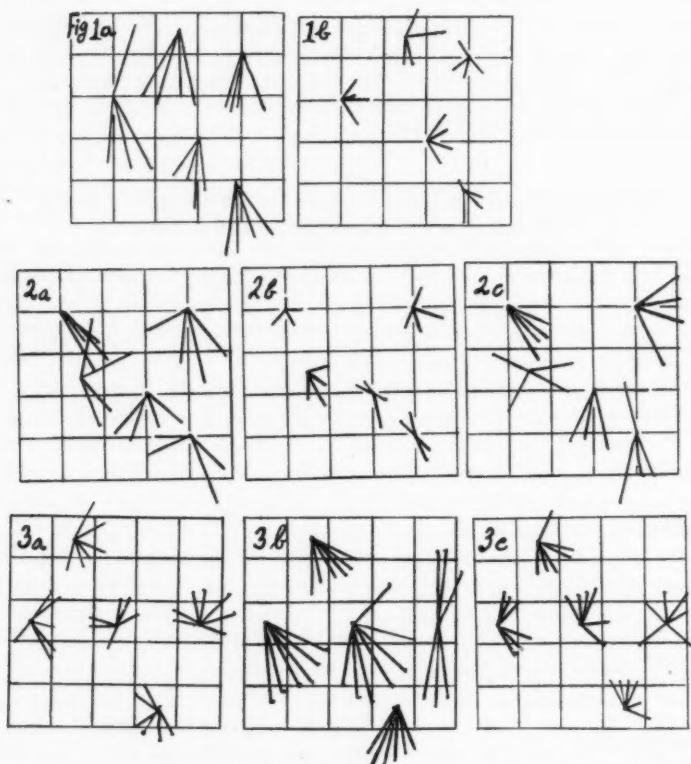


Fig. 1a. Localization of pain points under control conditions. b. Localization of pain points under irradiation conditions.

Fig. 2a. Localization of pain points under control conditions. b. Localization of pain points under irradiation conditions. c. Localization of pain points under control conditions.

Fig. 3a. Localization of pressure points under control conditions. b. Localization of pressure points under irradiation conditions. c. Localization of pressure points under control conditions.

RESULTS. I. *The influence of spinal irradiation upon the localization of pain points.* Figure 1 contains typical records of an experiment with pain points. The averages of the localization errors are contained in table 1.

From these records it is evident that under the irradiation condition the error of localization for pain points has decreased. The experiments were continued and the results summarized in table 2.

From an inspection of table 2 it is very clear that in every case under the condition of irradiation the localization error for pain points is greatly reduced. The decrease in error varies from 18 per cent to 62 per cent giving an average reduction of error of 35 per cent. Supporting these data are the verbal statements of the subjects showing that they are much more sure of their localizing under irradiation conditions, and that the stimulations seem to them to be more sharp and distinct.

That the irradiation effect is reversible is shown in figure 2. The averages are given in table 3. In these cases a third period of observation

TABLE 3

	CONTROL					IRRADIATION					CONTROL				
	Point A	Point B	Point C	Point D	Point E	Point A	Point B	Point C	Point D	Point E	Point A	Point B	Point C	Point D	Point E
Average.....	11.5	9.75	8.75	10.0	11.0	5.5	5.5	3.5	5.25	3.75	10.5	11.5	11.5	8.75	9.0

TABLE 4

	CONTROL					IRRADIATION					CONTROL				
	Point A	Point B	Point C	Point D	Point E	Point A	Point B	Point C	Point D	Point E	Point A	Point B	Point C	Point D	Point E
Average.....	6.5	6.9	5.2	6.2	6.5	10.9	14.5	13.7	14.5	11.3	6.9	6.5	6.3	6.5	5.5

was carried out several hours after the irradiation period to furnish a further control. Care was taken that the irradiation condition had entirely disappeared.

The possibility that the results may be caused by a change in the relative strength of the stimuli, inasmuch as the threshold is known (Goldscheider) to be lower under irradiation conditions, can be rejected on the basis of experimental results. Mayer has shown that the localization of pain points is independent of the strength of stimulations. The results of Mayer have been confirmed in this work. The conclusion is therefore imperative that the irradiation condition leads to a more accurate localization of pain points, due to qualitative changes in the pain sensations themselves.

2. *The influence of spinal irradiation upon the localization of pressure points.* Figure 3 contains typical records of an experiment with pressure points and table 4 summarizes the averages of localization errors. All ex-

periments on pressure points were effected in the order of control, irradiation, and control.

It is apparent that under the condition of irradiation the localization error for pressure points has greatly increased. Experiments were continued and the results are summarized in table 5.

Again do we observe the typical effect of lessened accuracy under irradiation conditions in every experiment, and again do the reports of the observers supplement the data. The stimulations were described by the observer as vague and uncertain. Many times the observer would have no idea of the location of the stimulus and would be unable to localize at

TABLE 5
Influence of spinal irradiation upon the localization of pressure points
(Summary of all experiments on pressure points)

SUBJECT	STIMULA- TIONS PER POINT	A ₁ CONTROL	B IRRADIA- TION	A ₂ CONTROL	$B - \frac{A_1 + A_2}{2} \times 100$
1 G (female)	6	4.1	8.2	5.0	82
2	6	3.8	11.7	2.5	266
3	6	5.0	12.5	4.8	155
4	6†		11.1	5.0	122
5 L (female)	4	12.0	16.2	12.3	33
6	4	13.9	16.5	12.6	24
7 H (female)	6	7.8	12.9	7.7	65
8	6	6.3	12.9	6.4	101
9 McC (male)	6	11.5	22.6	11.4	96
10	6	13.4	21.1	14.9	48

* Figures in this column were obtained by averaging the two control values and expressing the increase over this value under irradiation as a percentage thereof.

† (Same points as used in preceding sitting, but on the next day.)

all. With some of the points, repetition of the stimulus became necessary. Two of the observers reported that the hair felt as "large and flat across as the eraser end of a pencil." The great consistency of the results may be illustrated by experiments 3 and 4 in which the same points were stimulated over a period of two days. The results obtained in three control and two irradiation experiments check respectively with one another to a rather surprising degree.

It is known from the experiments of Goldscheider that the threshold for pressure points rises under the conditions of irradiation. Therefore, the question has to be decided as to whether or not the differences in the behavior of the pressure spots before and during irradiation are due to changes in threshold. To this purpose experiments were carried out with two of the observers. Stimulations in the control experiment were given just

above the threshold pressure, and in the irradiation experiment they were given with a pressure 46 and 77 times the changed threshold value respectively. The results of these experiments are summarized in table 6.

Table 6 indicates clearly that in spite of a great increase in the intensity of the stimulus the results are most comparable with those shown in table 5. Therefore, we may summarize the results of our experiments by saying that, independent of quantitative changes in threshold, the localization of pressure and pain points varies under conditions of irradiation. Consequently, the experimental facts must be explained on the basis of qualitative changes, in the respective sensations resulting from spinal irradiation. The existence of these changes became very apparent in the spontaneous remarks by the subjects which were mentioned above.

TABLE 6

The effect of increased strength of stimulation on the localization of pressure points under the condition of irradiation

SUBJECT	STIMULATIONS PER POINT		CONTROL			IRRADIATION		
	Experiment	Number	Average threshold	Stimu- lation used	Average local error	Thresh- old	Stimu- lation used	Average local error
			grams	grams				
McC (male).....	1	6	0.02	0.025	18.5	0.05	2.30	46.3
	2	6	0.02	0.025	10.8	0.05	2.30	18.8
	3	6			15.0	0.05	2.30	32.0
L (female).....	1	6	0.02	0.025	17.6	0.03	2.30	24.6
	2	6	0.02	0.025	9.5	0.03	2.30	23.5
	3	6			13.5	0.03	2.30	24.0

Out of the frequently observed fact of uncertainty of localization with pressure points under irradiation, there arose the question as to whether there is any change in the variability of the localization error under irradiation. Three of the subjects were given experiments in which each point was stimulated 25 to 30 times and the coefficients of variability were determined for both the control and the irradiation condition. The results are given in table 7.

One recognizes from table 7 that the variability increases very much on the average from about 60 per cent to 200 per cent when the irradiation condition is produced, thus indicating still further the great uncertainty of the observer in localizing pressure points under irradiation conditions.

The experiments described above show clearly that spinal irradiation exerts a specific influence on pressure and pain sensations. While the localization of pain points is improved, the pressure point localization be-

comes worse and more uncertain. It has been proved that these observations cannot be explained by threshold changes and therefore it seems probable that qualitative changes are brought about in pain and touch sensations which lead to characteristic changes in the accuracy of localization. An interesting physiological inference which can be drawn from our experiments concerns the theory of pain. As it is known, the question as to the independent nature of pain nerves is still in dispute. Goldscheider opposes von Frey's theory that pain is elicited by specific pain nerves. The experiments described in this paper would seem to support von Frey's theory since the sensations of both pain and touch undergo specifically different changes which become apparent in localizations under conditions of spinal irradiation.

TABLE 7

SUBJECT	V*				MEAN V	CONDITION
	Point 1	Point 2	Point 3	Point 4		
L (female).....	20.0	15.0	9.0	15.0	14.75	Control
	49.0	44.5	37.9	14.6	44.50	Irradiation
G (female).....	17.2	41.6	21.4	25.6	26.45	Control
	46.0	59.2	45.2	55.0	57.35	Irradiation
T (female).....	14.0	25.4	20.4	30.9	22.7	Control
	30.6	34.3	44.6	31.9	35.4	Irradiation

* $V = \frac{100 \sigma}{M}$, where $\sigma = \sqrt{\frac{\sum d^2}{N}}$ (Standard deviation); d means the deviation from the mean, N = number of stimulations, and M = the mean of the values.

The determination of the changes of the accuracy of localization under irradiation leave unanswered the question as to the behavior of the discrimination threshold under similar conditions. The latter has been examined by Schriever for pain sensations. He found an increase in the discrimination threshold. This shows that spinal irradiation influences localization and discrimination of pain sensations in opposite manners. It seems to indicate that the discrimination of two simultaneously stimulated points and the localization of a point on the skin involve two different mechanisms. Observations of Spearman and Schittenhelm have shown that this result holds also for touch stimulations under pathological conditions since one function may be greatly impaired independently of the other function. Schittenhelm found in a case where the spinal cord was injured, that discrimination of two touch points was almost impossible, although localization of one point was not impaired. The fact that spinal

irradiation also made apparent the mutual independence of discrimination and localization, as is the case in diseases of the spinal cord, supports our opinion that the study of spinal irradiation can profitably become the basis of an understanding of the patho-physiology of the spinal cord.

The experiments described in this paper, together with the observations of Goldscheider and Schriever, indicate the great influence of spinal irradiation upon the behavior of cutaneous sensations. Thus, it is no longer possible to assume that the influence of an alteration of the sensory part of the spinal cord is restricted to purely quantitative changes in the sensations according to the degree of irradiation. Although the mechanism of these changes is not known, the assumption seems probable that either the impulses originating in the peripheral sense organs can be modified while passing through the spinal cord, or that the spinal irradiation brings about a change in the reactivity of the cortical sensory center. Thus qualitative changes of the sensations will result.

SUMMARY

The localizations of pressure and pain points undergo characteristic changes when a spinal irradiation is brought about by pinching up a small area on the forearm. The accuracy in localization of pain points is improved while the localization of touch points is impaired. These changes are not produced by changes in threshold since they are independent of the intensity of the stimulus. The experiments indicate that an alteration of the sensory part of the spinal cord is inadequately described by hyper- or hypo-esthesia, because qualitative changes in the sensations which lead to quantitative deviations in the accuracy of localization are not taken into account. The importance of the experimental results for the physiology and patho-physiology of the spinal cord is briefly discussed.

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The electric mobility of oil droplets in soap sols and gels. HAROLD A. ABRAMSON.

It has previously been found¹ for soft (1 per cent) gelatin gels that no change in the electric mobility of microscopically visible particles occurs during the sol-gel transformation. The gels were stiff enough to prevent settling of the particles. This indicated that during the sol-gel transformation, no appreciable change in η , the viscosity, occurred with time. The apparent viscosity, η' , could be expressed

$$\eta' = f(F) + \eta$$

where F is the shearing stress and η a constant independent of time. Wyman's² measurements of the dielectric constant during sol-gel transformation lead to a similar conclusion.

The electric mobility of olive oil droplets in a soap (Lux) gel have now been investigated. Although η' may increase tenfold with a given shearing stress, little or no change in electric mobility of the droplets occurs. Gels can be obtained in which droplets remain suspended for days but which do not appreciably hinder their electric mobility. Stiff gels do hinder the electric mobility of the droplets with low field strengths. Certain novel features have been encountered. In $\frac{3}{4}$ per cent soap sols the electric mobility of the oil droplets approaches that mobility which the droplet would have in dilute alkali, nearly 8μ per second per volt per centimeter. This suggests that adsorbed hydroxyl ions are responsible for the high mobilities here observed and for the high transport numbers of soaps observed by McBain. It seems likely, therefore, that emulsification by soaps is accompanied by preferential adsorption of OH ions. We are inclined to the viewpoint that hydrolysis of a soap at an oil surface is accompanied by the adsorption of many (OH) ions, in addition to the soap ions.

The larger oil droplets open up in stiff gels soft channels into which smaller droplets migrate. And under certain conditions rows of small oil droplets in single file can be made to move in these channels which are seemingly liquid but surrounded by a rigid gel structure.

These data, as well as similar but less complete experiments which have been obtained with muscle globulin, are in further support of the viewpoint that electrical forces at play in living tissues are of a sufficient order of magnitude to account for the emigration of the leucocyte through the gel-like structure of the capillary wall.

¹ Abramson, H. A. Journ. Gen. Physiol., 1928, xi, 743.

Freundlich, H. and H. A. Abramson. Zeitschr. physik. Chemie, 1928, xlvii, 677.

² Wyman, J. Journ. Biol. Chem., 1931, xc, 443.

Electric mobilities of proteins in alcohol solutions. H. A. ABRAMSON and J. DANIEL.

Combining the theories of Helmholtz-Smoluchowski and Debye and Hückel it can be shown with certain assumptions that Q , the charge on a particle is,

$$Q = (K + K' \sqrt{\mu}) v, \quad (1)$$

where K and K' are constants, μ = the ionic strength, and v , the electric mobility. Assuming the charge of a protein to be proportional to its acid-base combining power, there results the rule: *In certain solutions of the same ionic strength the electric mobility of a soluble protein is proportional to its combining power for acids and bases.* This rule has been previously shown to be followed by dissolved egg albumin and gelatin and for surfaces composed of these proteins.¹ Unpublished data would indicate the validity of the same rule for crystallized serum albumin. These studies have been extended to solutions of alcohols. Although it has been found for aqueous solutions that v is proportional to the field strength, recent data of Ettisch and Zwanzig² would indicate that this is not necessarily true for glass in contact with alcoholic solutions. Gliadin, egg albumin and gelatin have, therefore, been investigated in various concentrations and kinds of alcohols. In all cases the mobilities of these proteins was proportional to the applied potential between 1 and 20 volts per centimeter. The study of protein mobility in alcohol solutions is, therefore, not complicated by the effect noted by Ettisch and Zwanzig. Equation (1) can therefore be tested in the presence of alcohols. These experiments are in progress.

Blood pressure as a chief factor modifying the water content of tissues. E. F. ADOLPH and M. J. LEPORE.

The arterial blood and the leg muscles of anesthetized dogs and cats were sampled at frequent intervals while the mean arterial and venous pressures were recorded. The pressures were varied acutely, either locally by partial occlusion of the dorsal aorta or inferior vena cava near the iliac junction, or generally by hemorrhage, transfusion, infusion, or intravenous administration of adrenalin or histamine.

When the arterial pressure was lowered the muscles lost in percentage water content, while the blood was greatly diluted as measured by dry weight, specific gravity, hemoglobin concentration, hematocrit, and plasma protein concentration. When the arterial pressure was increased either locally or generally the reverse occurred.

Local effects could be contrasted in the same animal, since during partial occlusion of the dorsal aorta the pressure was lower than normal in one half of the animal and higher than normal in the other half. Increases of venous pressure affected the distribution of water less than did the changes of arterial pressure, indicating that the induced changes of capillary pressure were less. Occlusions may have been accompanied by partial asphyxia of the tissues.

General pressure effects were pronounced when hemorrhage was produced, or when solutions were infused having both colloidal and non-

¹ Abramson, H. A. Proc. Soc. Exp. Biol. Med., 1929, xxvi, 689.

² Ettisch, G. and A. Zwanzig. Zeitschr. physik. Chem., 1930, cxlvii, 151.

colloidal osmotic pressures similar to those of blood. Even infusions of non-colloidal solutions affected the tissue water contents at first chiefly in parallel with changes of arterial pressure. In all experiments extreme changes of water content of muscle occurred within fifteen minutes, and usually with a lag of seven to ten minutes in their onset. The water content of the blood changed without any lag, and the fluid is believed to be located in other tissues before it appears in the muscle.

Measurements on the pressure of spinal fluid. E. J. BALDES and L. E. DANIELS.

Records of spinal fluid pressure are obtained by use of a special camera in which the sensitized paper moves slowly behind a long manometric glass tube. Light from a distant point source falls on the manometer. The liquid in the tube acts as a cylindrical lens focussing the light on the sensitized paper, thus recording the spinal fluid pressure. Directly in front of the manometer tube is placed the time marker and a Marey tambour so that the shadow of the pointer of each will be included in the record. The tambour is arranged to register the time of application of pressure to the jugular vein. Connection between the camera which has the manometer tube attached and the spinal puncture needle is made with a rubber tube with an adaptor on each end. All parts of the apparatus coming in contact with spinal fluid are carefully autoclaved.

Slides of records are presented which demonstrate: *a.* Determination of the initial pressure, showing changes in pressure due to respiration, pulse and patient's inconsistent relaxation. *b.* Effect of pressure on the jugular vein usually touch and heavy pressure on both sides and heavy pressure on each side separately. *c.* Effect of straining and bending the head backward and forward. *d.* Rapidity of response of the spinal fluid to applications of pressure to the jugular vein, to straining and to bending the head. *e.* Typical records of pathologic conditions such as spinal cord tumors.

Changes in the blood associated with fever induced by killed B. coli. M. GARCIA BANUS and E. GINSBURG.

Body temperatures of 40–41°C. were induced in dogs by a series of intravenous injections of a suspension of killed *B. coli*. The body temperature was maintained at a high level for longer than 10 hours and was then allowed to return to normal. The following changes were observed in the blood: 1. An increase in the concentration as determined by the percentage of total solids. This increase continued some time after the peak of the fever was passed. 2. An increase in the oxygen capacity and hemoglobin concentration. This increase was greater than could be accounted for by the increase in blood concentration. 3. A fall in the alkaline reserve not proportional to the change in body temperature. 4. An increase in the buffering power of the blood as indicated by a change in the slope of the CO₂ dissociation curve. This was not definitely related to the increase of blood concentration nor to that of the hemoglobin concentration. 5. The appearance of a non-functional pigment after several hours of fever.

Return to the normal value of all these factors occurred long after the disappearance of the fever.

Vasomotor changes induced by the direct application of heat or cold to the medulla. H. G. BARBOUR and F. JELSMA.

The effects of alterations in the temperature of the medulla have been studied by applying heat and cold directly to the floor of the fourth ventricle of the unanesthetized dog. Water of the desired temperature was passed through a small metal device giving a surface of about 6 sq. mm. which had been put in position aseptically under ether the day before.

When the medulla is thus heated by a small stream two or three degrees above the body temperature the ear temperature falls, indicating vasoconstriction. Cold on the other hand causes vasodilatation and warmth in the ear. Gradual increase in the intensity of the exciting agent may enhance the effects. Reversal of the direction of temperature change may however be ineffective until the body temperature level has been crossed.

Thus the medulla is affected by temperature in the opposite way from the basal ganglia, confirming our previous results where antagonism between carotid and vertebral circulations was observed.

Some postural deficiencies following certain cortical ablations. PHILIP BARD.

A cat that has survived bilateral removal of the neocortex over fourteen months shows approximately normal locomotion but when standing or sitting the legs tend to assume abnormal attitudes, e.g., abduction, retroposition of forelegs, forward extension of hindlegs, crossing of forelegs. Such abnormalities are in part due to deficient "placing" and "hopping" reactions of the legs.

Placing reactions. If a normal cat be held with chin on a table edge the forefeet are immediately raised, placed beside the jaws, and extended to a standing position. When forelegs or hindlegs are placed over a table edge they are at once brought up and placed for standing. If the animal be lowered slowly toward the floor the forelegs react so as to place the feet properly for standing.

Hopping reactions. When any leg of a suspended normal cat is placed in standing position and the trunk displaced forwards, backwards or laterally the leg hops in the direction of the displacement (Rademaker, *Rev. Neurol.*, 1930, 1, 337).

In the cat lacking neocortex and in cats which have long survived bilateral ablation of all cortex rostral to sulcus suprasylvius anterior the placing reactions of forelegs are absent, those of hindlegs exceedingly deficient, and the hopping reactions are absent or sluggish and incomplete. The same deficiencies are present but to a somewhat less extent, after bilateral ablations of only gyrus preoreus and sigmoid gyri.

Unilateral removal of gyrus preoreus and sigmoid gyri produces deficiencies in placing and hopping reactions of the contralateral legs, and after hemidecortication the deficiencies are slightly greater. Chronic unilateral thalamic and hypothalamic cats show contralateral abnormalities of about the same extent.

Unilateral ablation of all neocortex caudal to sulcus ectosylvius anterior produces no deficiencies of placing reactions and only a just perceptible deficiency of some of the hopping reactions of the contralateral legs.

Invariably associated with these abnormalities is an extensor hypertonia which is held in abeyance during walking or crouching but appears when any abnormal position is assumed or imposed.

Modification of the latency of warmth sensations by interference through paradoxical stimulation of cold end-organs. H. C. BAZETT and B. McGLONE.

The production of hot sensations through the simultaneous stimulation of both warm and cold spots appears to be generally accepted, but the fact that the latency of such hot sensations may be considerably longer than that for milder warmth (Altrutz) has received no explanation. Measurements of temperature changes in the prepuce correlated with temperature sensations have already been demonstrated to the society, as well as histological preparations which substantiate v. Frey's hypothesis that sensations of warmth are mediated by Ruffini's end organs. In the prepuce these end-organs occur commonly at a depth 0.3 mm. If these assumptions are accepted, estimates may be made of the time necessary to stimulate these end-organs and of the total change of temperature required. It is found that with weak stimuli (rates of change less than 0.3° per sec.) the latency is often long; with more rapid rates of change it is quite short, but with stronger stimuli which cause a change of 0.7° to 1° per second in the superficial tissues the reaction time is again longer, and the signals show evidence of subconscious hesitation. With still stronger stimuli the latency becomes somewhat shorter and the sensations begin to be called hot. With very strong stimuli the latency may be again somewhat increased, possibly as the result of vasodilatation. The most marked lengthening of the latency occurs with stimuli of moderate strength and is particularly in evidence in cold surroundings, i.e., under conditions where previous data suggest cold end organs are more readily excitable. The complicated curves for latency of sensation obtained with warm stimuli compared with the relatively simple curves found in the case of cold, suggest that paradoxical stimulation of cold spots is the rule, and that paradoxical stimulation of warm spots by cold is either impossible or extremely rare.

Polarization and action potential in nerve. G. H. BISHOP.

It is conventionally assumed that the same mechanism of polarization is involved both in the action potential and in the demarcation potential of nerve.

Since for a nerve impulse to be effectively recorded it must propagate, each active segment stimulating the adjacent segment, and since all depressants raise the threshold of stimulation, this decreased irritability might be expected to cause failure of conduction before complete depolarization is attained. This is found to be the case. The following agents cause blocking of about half the fibers of the frog sciatic responsible for the alpha wave at from two to three millivolts depression of resting potential in 30 minutes at 25°C ., when applied at one of two lead electrodes both on uninjured and equipotential nerve, stimuli one to two per second. The beta and following waves are depressed sooner in all cases. All agents were added to Ringer's solution and the pH adjusted to 8.4 (except CO_2).

KCl 0.3 per cent, veratrin 1/20,000, aconitin 1/200,000, N_2 1 atmosphere (lack of O_2 , Gerard's 1930 curve); CO_2 1 atmosphere does not block, but reduces action potential 50 per cent in amplitude with 1 millivolt depression of potential of rest.

Besides these agents *reducing* resting potential, others block with *increase* of 1 to 2 millivolts, in the following concentrations and the same time: cocaine 1/2,000, amyl alcohol 0.4 per cent. Na-Iodoacetate 1/5000 with stimulation in muscle.

It is found that if nerve is blocked by agents applied to otherwise normal tissue, the action potential is somewhat monophasic, when one electrode is on unaffected tissue below the block. If the nerve is not depolarized at the blocked region, the potential is strictly monophasic; if slightly depolarized it is partially monophasic.

With agents that depress the potential, the amplitude of action potential is reduced before blocking. With agents that increase the potential of rest, the evidence indicates that the individual axons fail to conduct with the action potential at full amplitude, apparently becoming refractory or non-irritable without previous decrease in energy output. Demarcation potentials from these nerves upon killing under one electrode are as high as twenty millivolts, or ten times the blocking potential. In the blocked region the threshold at time of block by any agent is two to three times normal.

Turning the latter procedure around, investigation of conditions at the killed end of a nerve indicates that the first response to a stimulus occurs at a point on the polarization potential gradient where the voltage is six or more millivolts below normal, and where the threshold is less than three times the normal. Presumably this is the point at which an impulse conducting into the killed region fails to propagate. Immediately after killing this point is very close to the dead region, then moves back toward normal nerve, and finally again moves forward toward the crush as recovery from injury takes place. The recovered region is more susceptible to block by depressing agents than before injury.

New data concerning acidity of gastric juice. EPHRAIM B. BOLDYREFF.

The subject of gastric secretion and the acidity of gastric juice has recently received a great deal of attention. There are two factors concerned in controlling the acidity of gastric juice, referred to in works devoted to the study of gastric secretion: 1, reflux of alkaline duodenal contents into the stomach, and 2, the supposed secretion of neutral chlorides. The author's observations on a number of dogs with Boldyreff's isolated stomach as well as on dogs with simple gastric fistula and Heidenhain's gastric pouch show that the secretion of pyloric juice is also of considerable importance. It was found that during prolonged hypersecretion of gastric juice a secretion of pyloric juice often takes place. The latter develops gradually and may sometimes reach a quantity as great as half of the total liquid contents of the stomach. Pyloric juice is a very faintly alkaline viscous fluid and invariably has the same chemical composition.

The careful investigation of the acidity of gastric juice obtained from isolated fundal portion of the stomach has convinced the author that there is no evidence to demonstrate the presence of neutral chlorides in the gastric juice.

Periodical passage of air from the stomach into the intestine (in fasting state, in man and animals). W. N. BOLDYREFF.

In my early work on the periodical activity I stated that during the periodical gastric contractions rumbling in the small intestine is heard.

It is evident that this is due to the presence of air which is forced by gastric contractions into the intestine from the stomach exactly at the moment when pancreatic juice and bile are flowing into the duodenum. Admixture of air with the fluid and the further progress of this mixture along the small intestine (duodenum and jejunum) cause a characteristic rumbling sound.

During periodical rumblings, each 24 hours, about 600 cc. of air enter the intestine from the stomach (adult dog).

Then how is the air supply restored in the stomach?

At times the registering apparatus, introduced into the stomach, indicates a negative pressure. Usually this occurs after strong periodical contractions and especially after long periods of work.

Of course, this condition is of short duration. Probably, in the movements of the neck and the trunk of the body, the esophagus admits some air from the naso-laryngeal cavity into the stomach, until the negative pressure disappears.

From this we conclude that in a fasting state, the air is constantly entering the stomach from the outside and it is forced by the stomach into the intestine where it is partly absorbed by the blood or lymph capillaries.

The work was carried out on an especially prepared dog for the measuring of the quantity of the intestinal air.

Comparison of the effect of single intravenous injections of thyroxine before and after thyroidectomy. WALTER M. BOOTHBY and C. M. WILHELMJ.

Wilhelmj and Boothby¹ showed that the calorogenic action of a single intravenous injection of thyroxine was not materially influenced by the administration of iodine. They found that the extra heat thereby produced up to the eighth day after the injection averaged 300 calories for three experiments without iodine, and 311 calories for two experiments with iodine.

Subsequent to these experiments the dog was thyroidectomized; the parathyroids were also apparently removed, as it has been necessary to keep the dog on calcium lactate and also at times on parathormone and cod liver oil. Under these conditions the extra heat formed following a similar intravenous administration of 10 mgm. thyroxine averaged 616 calories for 8 experiments. The values for each experiment are as follows: 505, 633, 373, 824, 730, 550, 644, and 666 extra calories for seven days inclusive.

Whether the increased calorogenic action is due to the lowered base line of heat production or to the increased irritability of the dog, as a result of the parathyroidectomy, cannot be definitely determined. It is possible that both factors played a part in the increased response, although the fact that the maximum elevation was maintained longer and then more slowly fell to the base line strongly confirms our previous observation on humans that with lower total concentration of thyroxine in the body it is less slowly destroyed or eliminated. This is in accordance with the exponential character of the decay curve of thyroxine, as previously shown by Boothby, Sandiford, Sandiford, and Baldes.²

¹ Wilhelmj, C. M. and W. M. Boothby. This Journal, 1930, xcii, 568.

² Boothby, W. M., I. Sandiford, K. Sandiford and E. J. Baldes. Trans. XII Int. Physiol. Congress, Skand. Arch. Physiol., 1926, xlix, 99.

The influence of motor nerve "fatigue" on the removal of a curare block by physostigmin. T. E. BOYD.

Shielded electrodes were placed on the intact hypoglossal nerves of the cat. Sufficient curare was given to produce a complete block. One nerve was left at rest, the other tetanized with slightly supramaximal stimuli at 100 per second. After 15 minutes the tetanization was stopped and a dilute physostigmin solution run slowly into a vein. The course of recovery from block on the two sides was compared, by alternate stimulation of the resting and the "fatigued" nerve with single shocks at 10 second intervals. The time required varied according to the depth of curarization and the rate of injection of physostigmin, but was always shorter for the tetanized than for the resting nerve. The prolonged stimulation therefore facilitates in some way removal of the curare block.

Since the circulation was intact, the difference in recovery might be due, not directly to stimulation of the somatic motor nerve fibers, but to vaso-motor effects from other types of fibers in the tetanized nerve. Vaso-constrictors from the cervical sympathetic are said to reach the tongue by way of the hypoglossal. If, however, both hypoglossals are left at rest in the curarized animal, and one cervical sympathetic trunk stimulated for 15 minutes, the subsequent slow injection of physostigmin first removes the curare block on the side *opposite* to the tetanized sympathetic. It seems unlikely, therefore, that sympathetic fibers are responsible for the effect described above. Other possible explanations are being investigated.

Observations on the cortico-adrenal hormone. S. W. BRITTON and H. SILVETTE.

Extracts of the adrenal cortex have been made according to the method of Swingle and Pfiffner, and their effects noted on cats, dogs, rabbits and rats. In a few cases tests on man have also been made. The extracts have been proved to contain significant amounts of the cortico-adrenal hormone, and corroboration is made of the results of Swingle and Pfiffner. The survival period of adrenalectomized cats is at least much prolonged, and may possibly be extended indefinitely, by daily injections of the extract. Treated animals gain in weight and appear normal. The extracts usually contain only one in two million parts of adrenalin, and the results are not produced by the adreno-medullary hormone. Particular attention has been given to recovery from the severe terminal symptoms of adrenal insufficiency. Evidences of resuscitation of adrenalectomized cats from extreme prostration following intraperitoneal injection of the Swingle-Pfiffner extract are apparent in 15 to 30 minutes: convulsions are abolished, the animals show an interest in their surroundings and attempt to sit up; within an hour or so they may walk about and appear practically normal, and two hours after injection they may take food. Probably of considerable importance are the increases in blood sugar to normal levels which are found to take place during the course of recovery. Usually it has been necessary to inject from 5 to 10 cc. of extract (1 cc. = 40 grams fresh whole gland) per kilo body weight of the animal, in the course of 24 hours, to effect restoration from the pronounced symptoms of adrenal insufficiency. These doses represent relatively huge amounts of cortical tissue—two thousand times or more than the amount present in the normal cat. Twenty cubic centimeters of the extract given intraperitoneally to a small (two-kilo) cat have produced no ill effects. The material is also non-toxic

when given subcutaneously, intramuscularly, intravenously, or intracardially. We have used the extract subcutaneously and intramuscularly in man without ill effect. The method of Hartman and his co-workers has also been used by us in the preparation of several batches of cortico-adrenal extract. This material, tested a great number of times on adrenalectomized cats, has given in only a few cases an indication that it contains in small amount the hormone of the adrenal cortex. The Swingle-Piffner extract is effective on cats by mouth. Dogs are stimulated to carry out two to three times as much work under its influence. Its injection produces sexual precocity in rats.

The discharge of impulses in the aortic nerve. D. W. BRONK and N. L. KALTREIDER.

Afferent impulses in the aortic nerve of the rabbit have been recorded by means of a vacuum tube amplifier and oscillograph simultaneously with the pulse wave. In each heart cycle there is a large outburst of impulses followed usually by inactivity. This discharge and the rapid rise in arterial pressure are concurrent. Frequently there is a second smaller group of impulses associated with the post-incisura rise. This evidence indicates that an important stimulus for the aortic nerve endings is a change of pressure. In support of this is the finding that if the character of the pulse wave is altered so as to make the rise of pressure less rapid there is a smaller impulse discharge (which, however, frequently lasts throughout diastole) even though the mean blood pressure is high. On the other hand, afferent impulses have been recorded when the mean blood pressure has been as low as 60 mm. Hg.

Pressure and the dynamics of cardiac muscle. DUGALD E. S. BROWN.

The response of the isolated auricular muscle of the turtle at temperatures from 0° to 7°C. is prolonged and the total tension is decreased when the muscle is subjected to a pressure of from 1000 to 6000 pounds per square inch. If, during the course of such a response, the pressure is abruptly decreased to atmospheric pressure, the muscle just as abruptly returns to the physiological state existing before the pressure is applied. The character of the response immediately adjusts itself to the physiological state determined by atmospheric pressure and, although at first the rate of development of tension is faster than in the normal response, the rate of the latter is rapidly attained. The tension developed after the decrease in pressure above the tension already existing in the response varies with the moment at which the decrease occurs. A decrease occurring at an increasing interval after the beginning of contraction results in a tension which increases with an increase in the interval, reaches a maximum early in the contraction, and then decreases along a logarithmic curve to approach the asymptote when the response is 95 per cent to 100 per cent completed. In a response at 3°C. and 5000 pounds pressure, having a duration of contraction of 4.5 seconds and a total duration of 18 seconds, the maximum is attained in 0.4 second. A decrease in pressure during the latent period results in a normal contraction apparently unaffected by exposure to the high pressure. If in this experiment the ratio of $\frac{H}{T_1}$ for cardiac muscle remains constant at atmospheric pressure, the tension resulting from a decrease in pressure may be considered as a direct measure

of the potential energy of the contracting muscle at the moment when the decrease occurs. The curve of the tension produced following a decrease in pressure at successive intervals throughout the response represents, therefore, the course of the process of building up and degradation of potential energy associated with the development of tension in the muscular response under pressure. It has not been established whether the potential energy existing at any moment may be attributed chiefly to the concentration of some component responsible for the development of tension or to the viscoelastic properties of the muscle.

Calcium as a normal constituent of the sweat. J. E. BRYANT and G. A. TALBERT.

A simultaneous study was made of the blood calcium and sweat calcium. The colorimetric method of Roe and Kahn was used, which was discovered to check very favorably with the micro-chemical method of Kraemer and Tisdall. Eighty-three determinations were made in which, without exception, calcium was found as a constituent of sweat varying from 5 to 10 mgm. per 100 cc. at times approaching quite closely to that of the blood, but more frequently, about 2 milligrams less than the blood. The effect of sweating in blood calcium was inconstant, yet more frequently it was reduced.

Relative rates of glucose and levulose absorption. G. E. BURGET and PHILIP MOORE.

Two methods of investigation were employed and Pfanstiehl sugars of specific rotatory powers used. 1. Chronic closed intestinal loops in dogs (Burget, Martzloff et al., Arch. Surg., 1930, xxi, 829). The dogs were in good condition with loops which we had every reason to believe were normal. After washing out the loop with normal saline, 1.5 to 2.0 grams of the sugar in solution were introduced by a hypodermic needle and syringe. At the end of an hour the loop content was aspirated and the amount of sugar determined. Fifteen observations were made with each sugar without any wide variations and with practically no difference in the rates of absorption. 2. Rabbits in which loops of intestine were tied off under anesthesia. Two loops, one for each sugar, were used in each animal. After closing the abdomen the animals were kept warm while recovering from the anesthetic. At the end of an hour they were sacrificed and the contents of the loops recovered. Observations on a number of rabbits have been uniform and failed to show any difference in the rates of absorption of the two sugars.

Respiratory quotient, alveolar air and dead space before and after the ingestion of glucose and fructose. THORNE M. CARPENTER and ROBERT C. LEE.

A series of determinations of the alveolar air intermittently and of the respiratory exchange continuously on a trained subject led to the conclusion that it is feasible to use the alveolar air as a control upon the normality of breathing and upon the accuracy of the respiratory quotient as an index of the character of the metabolism. The results of experiments in which 25 grams of glucose or of fructose were given to the subject showed that there was no significant alteration in the alveolar carbon dioxide as the result of the ingestion of these sugars. The conclusion was drawn that the respiratory quotients after the ingestion of these sugars were true

indices of the character in the metabolism and that the higher respiratory quotient after fructose than after glucose was not due to overventilation. A series with an untrained subject showed that even in a condition where no sugars were given there was a variable alveolar carbon dioxide with a simultaneously variable respiratory quotient. The sugars produced the same sort of change in the alveolar air, but it was found feasible to make a correction of the respiratory quotient after fructose or glucose for overventilation, and when this was done, the rise in quotients with the untrained subject was nearly the same as with the trained subject. The alveolar respiratory quotients parallel in the main the course of the quotients of the expired air and therefore, if sufficient number are obtained, they may be used as an index of the character of the metabolism. Utilization of the various measurements for the calculation of the effective dead space led to the conclusion that the dead space varied with the depth of respiration, and for these two subjects constituted a flat percentage of the volume of respiration. This finding is of value in the case of the trained subject because the alveolar air can now be calculated upon the past experimental work on the effect on the respiratory exchange of the ingestion of sugars.

Metabolism of ingested protein and carbohydrate in the fasting dog. WILLIAM H. CHAMBERS and MARGARET DANN.

In previously reported experiments on "hunger diabetes" in dogs no oxidation of carbohydrate was observed after the ingestion of 50 grams of glucose, but increasingly better utilization of the sugar was found with successive daily doses. The following results have been obtained in a further study of fasting dogs in the respiration calorimeter. The administration of 75 grams of glucose produced essentially the same improvement in carbohydrate metabolism when given at one time or in three doses at 4 hour intervals. When 77 grams of gelatin, which would yield about 40 grams of glucose in the phlorhizinized dog, were ingested by fasting dogs there was little rise in the blood sugar concentration and no glycosuria. In one dog the R.Q. fell from 0.72 to 0.69 after the first administration of gelatin, indicating that there was no oxidation of sugar derived from gelatin. The heat production rose about 30 per cent. Following a second dose the R.Q. increased from 0.71 to 0.77. Gelatin reacted like glucose in improving the utilization of carbohydrate given the following day.

Earlier experiments have shown that injected insulin would not effect an immediate complete recovery of sugar metabolism. The addition of 2 grams of Liebig's beef extract to 50 grams of ingested glucose or a meal of 200 grams of raw meat 18 hours previously had no marked effect on the metabolism of ingested sugar. A large amount of glucose mixed with cracker meal, about 300 grams of carbohydrate in 2 days, was efficacious in promoting an almost exclusive oxidation of sugar when 50 grams of glucose were given on the morning of the third day. However, in a similar experiment with glucose alone, 375 grams during a period of 4 days, the non-protein R.Q. was raised to only 0.93. These results suggest that in addition to the insulin deficiency the lowered glycogen stores in the body rather than a lack of intermediary extractive or nitrogenous materials are responsible for the absence of complete carbohydrate metabolism in "hunger diabetes."

The action of dyes, cyanide and carbon monoxide on nerve respiration.

T. H. CHANG and R. W. GERARD.

We have previously shown for vertebrate and invertebrate nerve that methylene blue (or cresyl blue) markedly increases the resting oxygen consumption, up to doubling, while cyanide largely abolishes it. The cyanide inhibition can be in part or completely reversed by addition of a dye.

A further series of 14 experiments with cresyl blue solutions well neutralized and buffered has given an average increase of the resting oxygen consumption of frog nerves of about 100 per cent, somewhat more when the sheath is split (21–24°C.) At pH 7.8 the optimal concentration for a five-hour period is 0.03–0.035 per cent; more concentrated solutions of the dye give greater initial increases which fall off rapidly; weaker ones give lesser increases but better sustained. In 12 experiments the more strongly oxidizing dichlorophenol-indophenol gave only an 18 per cent average increase. The reversal of cyanide inhibition by cresyl blue has also been obtained on frog nerve. Light has no influence on respiration in presence or absence of the dyes and/or cyanide.

In 98 per cent CO, 2 per cent O₂, the resting respiration in the presence of cresyl blue is depressed 70 per cent in the dark, less than 50 per cent in the light (a 200-W metal filament lamp, 4–15 cm. distant). The average values for 30 experiments are: In air, 17, in CO dark, 5.5, in CO light, 8.7. The same nerves in CO before addition of the dye gave: in the dark, 4.1, light, 7.8. The addition of cresyl blue led, therefore, to a small but quite regular increase in respiration in CO. This is, however, much less than the rise it can induce with the nerve in simple buffer or in the presence of cyanide. The actions of the systems cyanide-dye and carbon monoxide-dye on the respiratory mechanisms thus appear to be not identical. This is further indicated by the action of dyes on respiration in the simultaneous presence of monoxide and cyanide. Similar experiments with 97 per cent CO and controls with 97 per cent and 98 per cent N₂ demonstrate an adequate oxygen supply.

Preliminary experiments with yeast indicate a somewhat different situation. Although methylene blue is inactive, cresyl blue doubles the respiration in phosphate buffer; glucose increases it 15 times. Ninety-eight per cent CO does not affect the respiration in plain buffer nor the increase due to the dye, while abolishing that due to glucose. Cyanide cuts the respiration in buffer to a third and addition of methylene blue leads to no increase. In the presence of glucose, respiration is cut by cyanide to a value below that in simple buffer, but this is still twice the rate in buffer and cyanide but without glucose.

The oxygen consumption of washed muscle brei and sodium succinate is inhibited by cyanide or monoxide (occasional stimulating effects by the latter) and is restored by cresyl blue.

The action of bufagin and marinobufotoxin, obtained from the secretion of Bufo marinus (Bufo aqua). K. K. CHEN, H. JENSEN and A. LING CHEN.

The digitalis-like action of *bufagin* was already shown by Abel and Macht (Journ. Pharm. Exp. Therap., 1911–12, iii, 319). Our electrocardiographic studies confirm their findings. The average fatal dose in cats was found to be 0.50 mgm. per kgm. The minimal emetic dose in pigeons was determined to be 0.375, and that in cats approximately 0.225

mgm. per kgm. Like cinobufagin, its action is not persistent. *Marinobufotoxin* is a new principle isolated from the secretion of the Jamaica toad. Its action is similar to that of cinobufotoxin as shown by electrocardiograms. The average fatal dose in cats was found to be 0.49 mgm. per kgm. The minimal emetic dose in pigeons was estimated to be 0.25 mgm. per kgm. *Marinobufotoxin* has a more emetic action than bufagin.

The action of the principles of Ch'an Su, the dried venom of the Chinese toad.

K. K. CHEN, H. JENSEN and A. LING CHEN.

Pharmacological studies were made with six principles isolated from Ch'an Su. A. *Cholesterol* has an ergosterol content of 2 parts per thousand. The amount of ergosterol was proved both spectroscopically and biologically. B. *Epinephrine* is identical with that found in adrenal glands and the secretion of the Jamaica toad. C. *Cinobufagin* has an action similar to that of G-strophanthin as shown by electrocardiograms, myocardiograms and the emetic action. Its average fatal dose in cats (Hatcher's method) was determined to be 0.2 to 0.3 mgm. per kgm. Its minimal emetic dose in pigeons was found to be 0.3, and that in cats 0.125 mgm. per kgm. The cardiac action is not persistent in contrast with that of digitalis glucosides. The principle has also a local anesthetic effect, stimulates the medulla followed by a depression in frogs, and increases the tone of smooth muscles of the blood vessels, intestine, and uterus. D. *Cinobufotoxin* has an action similar to that of cinobufagin. The average fatal dose in cats was estimated to be 0.35 mgm. per kgm. The minimal emetic dose in pigeons was determined to be 0.2, and that in cats 0.125 mgm. per kgm.—cinobufotoxin being more emetic than cinobufagin. The principle is also rapidly eliminated. It has a stimulating action on the smooth muscles of blood vessels, intestine, and uterus. E. *Cinobufotenine* in the form of a flavanate has a marked pressor action owing to the vaso-constriction and cardiac stimulation, being equivalent to about one-tenth of the activity of epinephrine. It increases the tone of isolated intestines and uterus. F. *Suberic acid* is relatively much less active than the other five principles. It inhibits the contractions of intestine and uterus.

The active grouping in the molecules of cinobufagin and cinobufotoxin.

K. K. CHEN, H. JENSEN and A. LING CHEN.

In order to find out which part of the molecule of cinobufagin is important for the characteristic action we studied pharmacologically the following derivatives. A. *Acetylcinobufagin*, B. *Cinobufagien*, C. *Cinobufagone*, a mono-ketone of cinobufagin, D. *A chlorine derivative of cinobufagin*, and E. *Cinobufaginic acid*, formed by the opening of the lactone ring. The frog's heart perfused according to Howell and Cooke's method, the cat's blood pressure, and the rabbit's isolated intestines were used as indicators of physiological activity. It was found that compounds A, B, C, and D all caused systolic standstill of the frog's heart, rise of blood pressure, and increase in tone of intestinal contractions. Cinobufaginic acid is practically inactive in the same dosage. Since cinobufaginic acid is the only compound devoid of the lactone group, it is provisionally concluded that this group, the lactone ring, is responsible for the physiological action.

Suberic acid and arginine in the form of a flavanate, the decomposition products of cinobufotoxin, were studied in similar manner. They do not

have the typical action of the latter. Since our formula for cinobufotoxin is derived by adding 1 molecule of suberyl-arginine to 1 molecule of cinobufagin, it becomes obvious that the activity resides in the cinobufagin component.

Placental hormones. J. B. COLLIP, D. L. THOMSON, J. S. L. BROWNE, M. K. MCPHAIL and J. E. WILLIAMSON.

Extracts prepared from fresh human placenta by extraction with one and one-eighth volumes of acetone have been separated into various fractions by the use of alcohol, ether and acetone, and these fractions have been studied both physiologically and chemically. As a result of a very large series of animal experiments, it may be concluded that at least three active substances are contained in the original acetone extract. The ether-soluble fraction has been shown to contain oestrin. After repeated extraction with ether from acid aqueous solution the aqueous phase has been shown to contain two oestrogenic substances. One of these is active when fed, the other only when injected. The former substance is effective in minute amounts when fed to immature female rats. Much larger doses may produce oestrus in adult castrates, but this may be due to traces of oestrin in such extracts. The latter, which is the anterior-pituitary-like hormone of the placenta, has been purified to a high degree and extensive observations have been made on its physiological effects.

Cardio-vascular changes in cats during experimentally produced convulsions, following section of the vagi and stellates. HELEN C. COOMBS.

The technique of the injection of camphor monobromide, the convulsant agent used, has been described previously.¹ The stellates were excised without rupture of the pleura. The vagi were divided in the neck. A control record was taken of the blood pressure and heart rate in the otherwise normal cat, before, during, and after the injection of a sub-maximal dose of camphor monobromide. Following this, the stellates were removed, or the vagi divided under anesthesia, the wound was moistened with novocaine and the ether intermitted. In about fifteen minutes, camphor monobromide was again injected while a continuous tracing of blood pressure and respiration was taken. The following table gives the variations in blood pressure and heart rate shown under the different conditions.

	BEFORE CONVULSION		DURING CONVULSION		AFTER CONVULSION	
	Heart rate	Blood pressure	Heart rate	Blood pressure	Heart rate	Blood pressure
Control.....	200	110	210	130	192	100
After section of vagi.....	228	120	228	180	222	110
Control.....	216	190	224	205	200	185
After excision of stellates.....	180	120	240	125	225	100
After section of vagi (first convulsion).....	228	120	228	120	198	90
Second convulsion.....	216	70	216	70	200	60

¹ Wortis, S. B., H. C. Coombs and F. H. Pike. 1931. Arch. Neurol. and Psychiat. (in press).

The table shows that

1. After division of the vagi alone, heart rate tends to remain constant, while blood pressure rises during the convulsion.
2. After division of the stellates alone, heart rate rises, but there is no great increase in blood pressure during the convulsion.
3. After division of both vagi and stellates, neither the rate of the heart nor the blood pressure is greatly affected by the convulsion. There is a progressive fall in blood pressure as the convulsions continue, and the animal succumbs much earlier than the control.

The combined effects of occlusion of the head arteries and the intravenous injection of absinth or camphor monobromide in cats. HELEN C. COOMBS and F. H. PIKE.

The technique of occlusion of the head arteries and the technique of injection of the convulsant agent were the usual routine we have followed in previous work.

1. When the convulsant agent was injected in sub-maximal doses before the occlusion of the head arteries, the results, as stated below, varied according to the interval elapsing between the two procedures.
 - a. When the occlusion followed injection within twenty seconds, no real convulsion occurred, and the blood pressure curve was of the usual form in anemia.
 - b. When the occlusion followed the injection within forty to fifty seconds, convulsions due to the drug ceased within twenty to thirty seconds after the occlusion, but the blood pressure rose to a greater height than with either procedure alone.
 - c. When the occlusion followed the injection after more than sixty seconds, convulsive movements usually persisted up to forty-five to sixty seconds, and again the blood pressure rose to the greater height.
 - d. When the occlusion was done at the end of the convulsion (about two minutes after the injection of the convulsant agent) the ordinary cardiovascular response occurred, but the blood pressure did not rise so high as in (b) or (c), and its persistence at a high level was markedly less than in the control.

The curve of response to occlusion appears to be superposed on the curve of response to the convulsant drug much as muscular contractions may be superposed in the ordinary laboratory experiment.

2. When the order was reversed, and the injection of the convulsant agent followed close upon the reestablishment of the cerebral circulation, there was sometimes a considerable rise of blood pressure when there was no visible response of the skeletal muscles. Intravenous injection of curare following such a rise resulted in a grave fall of blood pressure. A second occlusion was often effective in producing a second anemic rise of pressure at a time when camphor monobromide failed to elicit any visible reaction.

The relation of the minimal convulsive dose of absinth or camphor monobromide to conditions of high and low blood pressure in cats. HELEN C. COOMBS and F. H. PIKE.

It has been previously observed that intravenous administration of adrenalin lowers the minimal convulsive dose of absinth in the cat (Pike and Notkin, 1930). We have observed also that mechanical compression

of the abdominal aorta resulting in a rise of systemic blood pressure, just following a dose of the convulsant agent which was below the control minimal convulsive dose, will elicit a clonic convulsion.

In the present series of experiments, in which a continuous record of blood pressure was taken, after finding the minimal convulsive dose of the convulsant agent, sodium nitrite was given intravenously. The control dose of absinth or camphor monobromide was again injected, and failed to elicit clonic convulsions. A higher dose was necessary before the clonic convulsion appeared. Pike and Notkin (unpublished results) also observed that, after the intravenous injection of sodium nitrite, a larger dose of absinth was necessary to elicit a convulsion.

It appears that among other possible conditions which affect the susceptibility of an animal to a convulsant agent, blood pressure must be reckoned as one. The susceptibility varies inversely as the mean blood pressure.

The regulation of blood lipase and diastase by the liver. LATHAN A. CRANDALL, JR. and IAN S. CHERRY.

We have previously shown that in experimental and clinical liver damage, in experimental and clinical pancreatic injury, and in multiple sclerosis an olive oil splitting lipase appears in the blood, and that this lipase is not present in normal blood from dogs or human beings. If this lipase is steapsin from the pancreas, the blood diastase might be expected to show similar changes.

Ligation of the common bile duct in 7 dogs has been followed within 24 hours by the appearance of large amounts of an olive oil lipase in the blood; diastase rises more slowly, reaching about double its normal value on the second to fourth day. These changes in the blood enzymes are not due to exclusion of bile from the intestine since bile fistula dogs show no lipase and no increase of diastase until a hepatitis results from ascending infection.

Blood from Eck fistula dogs usually contains an olive oil splitting lipase, although this enzyme tends to disappear on starvation. Blood diastase in these animals shows greater fluctuations than in normal dogs, frequently being well above the normal limit. The injection of secretin (6 times in 4 dogs) into Eck fistula animals has always been followed by a rise in blood diastase and in every case but one by the appearance of an olive oil lipase or an increase in amount of this lipase if it were already present.

A comparison of enzymes in the portal, saphenous, and arterial blood has been made in unanesthetized dogs with London cannulae on the portal vein. Lipase alone was studied in 2 dogs, both lipase and diastase in 3. In one animal in the fasting state lipase was present in the portal blood but none in blood from the saphenous vein. In fasting dogs the diastase in the portal vein blood is usually slightly higher than that in saphenous blood. Six injections of secretin in 3 of these animals resulted in all but one case in the appearance of olive oil lipase in the portal but not in the saphenous blood and an increase in portal blood diastase above that found in the systemic blood. Variations between saphenous and arterial blood were within the limits of error.

Pancreatic juice was injected intravenously (30 cc.) in one dog with a London cannula on the portal. Following the injection olive oil lipase appeared in all samples but was consistently higher in portal than in

saphenous blood. Blood diastase was increased 600 per cent and was also consistently higher in the portal than in the saphenous specimens.

Three dogs were subjected to simultaneous pancreatectomy and ligation of the common bile duct. The diastase in these animals promptly decreases as after simple pancreatectomy. Small amounts of olive oil lipase appeared in the blood of all three dogs but the amounts present were much smaller than in common duct ligation alone. A similar small amount of lipase appears after pancreatectomy without common duct ligation and then falls to zero; it probably originates in minute fragments of pancreas which are not removed.

The evidence suggests that these enzymes are absorbed from the gastrointestinal tract, and that the olive oil lipase (steapsin) is entirely and the pancreatic diastase partially removed from the blood by the liver.

Report of progress of research into the physical nature of cells (no conclusions are drawn). GEORGE W. CRILE, MARIA TELKES and AMY F. ROWLAND.
Cells were formed in a mixture of brain lipid, the protein of any organ, and brain ash (electrolyte) solution.

These cells are designated autotrophic cells.

The lipid was extracted with ether from dried fresh brain.

The protein was extracted with salt solution from the residue remaining after the lipid was extracted.

The brain ash (electrolyte) solution consisted of a solution of distilled water of various salts in the proportions in which they are present in the brain.

No cells preëxisted in the sterile solutions used.

Continued measurements for 10 days on autotrophic cells showed production of urea.

The effect of anoxemia on the digestive movements of the stomach. GEORGE CRISLER and E. J. VAN LIERE.

Enterographic records of the digestive movements of the stomach were made in barbitalized dogs fed one hour previously. Varying degrees of anoxemia were administered. Oxygen tensions below 10 per cent inhibited the movements in all animals. Some were affected by tensions as high as 12 per cent. The inhibition consisted of a decrease in amplitude, a decrease in tone or both. It seems that the fall in tone is present only in cases where the initial tone is high. If it is low the inhibition is manifested only in the decreased amplitude. The fall in tone is in contradistinction to the rise in tone seen in hunger contractions. In all but one dog the contractions could be completely inhibited after varying periods with oxygen tensions lower than 7 per cent. Upon re-oxygenation there was frequently a period of greater than normal activity as evidenced by increased amplitude, increased tone or both. The stimulating action came on very promptly without a quiescent period as seen in hunger contractions. In no experiment were any signs of vomiting or retching seen. Tracings were not included in which skeletal movements were apparent, or in which the animal showed extraordinary respiratory stimulation during anoxemia. It appears that anoxemia causes effects on digestive movements of the stomach, as indicated by enterographic methods, practically opposite to those on hunger contractions, as indicated by the balloon method. Correlation tracings of the digestive movements by the enterographic and the balloon methods are being made.

Studies on the carotid sinus. S. P. CROMER.

In a series of 21 dogs the presence of reflexes from the carotid sinus which affect blood pressure, heart rate, respiration and coronary flow has been verified under barbital anesthesia. Some animals were studied with only the sinuses exposed; some with vagi cut or stellates removed; others both with stellates removed and vagi sectioned. Blood pressure and respiration records were taken.

The following results of Hering on the carotid sinus reflex were verified in acute experiments: a. Digital pressure on carotid sinus (intact animals) slows the heart. b. Effective stimuli for release of the reflex are: 1, mechanical stimulation of the common carotid; 2, mechanical and electrical stimulation of the carotid sinus or its nerve. c. The above stimuli are ineffective after denervation of the carotid sinus. d. Strong stimuli tend to decrease heart rate and blood pressure. e. Mechanical stimulation of vagus does not release the reflex. f. A reflex change of respiration is usually obtained.

The following results of Hering and Danielopolu and their co-workers upon carotid sinus reflexes were verified in acute experiments: a. After vagotomy or removal of the stellates stronger reflexes result upon stimulation of sinus. b. After vagotomy blood pressure is lowered although the heart rate does not show a similar change. c. Rate of coronary flow (Morrowitz cannula) is affected, possibly as a result of blood pressure changes. d. Paroxysmal tachycardia in man is stopped by digital pressure on the carotid sinus.

The author disagrees with Danielopolu in that: a, removal of stellates and vagotomy in acute experiments does not entirely remove the reflex effects on heart rate and blood pressure, although it does lengthen the reflex time; b, the electrocardiogram is not modified by stimulation of carotid sinus after removal of stellates in chronic experiments.

On five dogs blood pressure, heart rate and respiration were taken under basal conditions. After vigorous exercise readings were again taken for fifteen minutes at five minute intervals with no changes. Following denervation of the sinuses similar records were taken. If the carotid sinus plays a rôle in the physiology of blood pressure, heart rate and respiration, it is easily taken over by other mechanisms.

Ossification in the absence of the parathyroid glands. CARL A. DRAGSTEDT.

Experiments have been performed showing that bone repair after thyroparathyroidectomy in dogs is delayed, confirming the reports of many investigators in this respect. Bone repair goes on to completion, however, and the delay becomes significantly less if calcium administration is adequate, indicating that the parathyroid hormone is not indispensable for the processes of ossification. This is more clearly indicated in experiments on ectopic bone formation. Bone will form in fascial transplants in the wall of the urinary bladder in thyroparathyroidectomized dogs if adequate calcium is given, while it does not form if such dogs are maintained with low blood calcium levels.

The effect of diverting adrenal vein blood into the portal vein on blood sugar.

LESTER R. DRAGSTEDT and JAMES C. ELLIS.

An anastomosis was made between the vena cava and portal vein in 11 dogs. The vena cava was ligated central to the anastomosis. This

produced a temporary diversion of adrenal vein blood into the portal vein and caused a hyperglycemia of greater extent and longer duration than control operations. An anastomosis similar to the one described above was made in 4 dogs and the vena cava ligated. Subsequently by means of several stage operations, both lumbo-adrenal veins distal to the gland were tied off and the vena cava again tied off this time below the entrance of the renal veins. This operation produced a permanent diversion of adrenal vein and renal vein blood into the portal vein. The blood sugar remained above normal for from 30 to 90 days and the daily variations were much wider than those obtained in normal animals. Glycosuria was not produced.

The effect of sodium amytal on the blood sugar level in the albino rat and the dog. M. DRESBACH and F. S. RANGLES.

In a series of 164 determinations of the blood sugar on a total of 151 normal, non-fasting rats, of both sexes and 3 to 9 months old, the average was 107.5 mgm. per 100 cc. (Randles-Grigg modification of the Folin-Wu macromethod and using 0.1 cc. of tail blood.) In 61 tests on 40 similar rats in fairly deep, or profound, anesthesia by 60 mgm. of amytal per kgm. the average was 108.4 mgm. over a period of about 2 hours.

In 22 tests on 6 adult dogs, of both sexes, and fasted for 24 hours, the average before anesthesia was 90.9 mgm. During a period of 1 to 6 hours of anesthesia by 30 to 70 mgm. of amytal per kgm. the average of 52 tests was 91.7 mgm. per 100 cc. (Folin-Wu macromethod with vein blood. The amytal was generally given by vein.)

The experiments therefore showed that during amytal anesthesia, more or less profound, the average blood sugar content was unchanged in a group of 40 rats and in six dogs, kept on ordinary standard diets. There may, however, be marked individual variations in the blood sugar level during amytal anesthesia. In some individual rats there may be a fall, instead of a rise, but the average in a large series is but little affected. In dogs we have seen similar individual variations, with and without amytal anesthesia. These experiments do not indicate the reason for the conflicting results obtained by other observers. No particular effect of 24 hour fasting was seen as regards the general result in the rats used in the experiments here reported.

The identity of lymph and tissue fluid. CECIL K. DRINKER and MADELEINE E. FIELD.

Experiments carried out during the past two years have convinced us that permeability of capillaries to the blood proteins is a more general property than impermeability. Lymph collected from many different sources in the same animal contains from 0.4 to 4.5 per cent of protein. This consists of serum albumin, serum globulin and fibrinogen and has an osmotic pressure of around 165 mm. of water, less than one-half that of normal blood serum. It must come from the blood since there is no evidence that tissues in general, or the lymphatic endothelium, can form it.

The extraordinary ease and promptitude with which large molecules and particles enter the lymphatics but not the blood capillaries in the great subcutaneous areas incline us to the belief that tissue fluid and lymph are identical and that the lymph promptly contains all material from the tissue spaces which is either unabsorbable or slightly absorbable by the blood

vessels. The actual transudate from the blood vessels probably contains the blood proteins in higher dilution than does the tissue fluid, which is subject to concentration by the withdrawal of water into the capillaries and to dilution by rapid filtration of water. Lymph taken from a cervical lymphatic probably represents the average tissue fluid composition of the region drained by a large group of lymphatics, each of the microscopic lymphatics in the area drained contributing the proteinized tissue fluid available at the moment.

Relation of hysterectomy of long standing to voluntary activity in the white rat. E. P. DURRANT.

Thirteen hysterectomized and eleven control animals about 90 days old were kept in recording activity cages for 16 months. The uninterrupted rhythmic increase and decrease in voluntary activity, heretofore shown to be correlated with the oestrous cycle, indicate that the cyclic changes in the ovary of the white rat are independent of any hormonal influence from the uterus.

The irritability of nerve in response to electrical currents, with special reference to the nature of the relatively refractory phase. JOSEPH ERLANGER and EDGAR A. BLAIR.

Gildemeister and Kato, by indirect stimulation of amphibian muscle, showed that the 0.4 to 2.0 ms. period of enhanced irritability produced by a subthreshold shock is succeeded through 3 to 10 ms. by a period of subnormal irritability. These findings are confirmed. In addition, it is shown that the summation interval is longer than, and, within limits, independent of, the duration of shocks of known form, and, with the same shock, is longer in polarized than in normal nerve. Furthermore, while Q_{10} of induction shocks and of their artefacts is about 1, that of the summation interval may fall between 1.3 and 1.7. The subsequent period of depression approaches the duration of the relatively refractory phase at temperatures above 12°C. Reversing the direction of the activating shock reverses the irritability picture. An anodal shock delivered during the absolutely refractory phase may almost abolish the relatively refractory phase.

At the cathode of a relatively long subthreshold constant current the irritability on the make first rises to a plateau and then, with a sharp discontinuity, falls to the level determined by polarization. Then, the summation interval is longer, and also the latent period, at least in most of the fibers, than in normal nerve. The lengthened latent period accounts for an apparent preterminal fall in irritability on the break, lasting about 0.5 ms. at 12°C., and also for the shift that occurs, in cathodally polarized nerve, of the crest of the simple action potential, uncondacted and conducted, into a later position in the curve, assuming that at the same time both of the phases are shortened. The preterminal fall is followed by a short summation interval and this by a period of post-cathodal depression, again about as long as the relatively refractory phase.

The irritability at the cathode of a current growing at less than the liminal rate first rises and then falls. In the latter stage the nerve gives every evidence of being in a state of cathodal depression.

The effect of Janus green on blood vessels. Preliminary report. G. HAROLD ETTINGER.

The vital stain Janus green has, in small doses, a very powerful pressor effect upon the blood vessels of the frog, guinea pig, cat and rabbit. This has been demonstrated in perfusion experiments, in blood pressure determinations under ether and urethane, and by intravenous injection without anesthesia. The effects in mammals are very noticeable in the pulmonary vessels, and are more easily demonstrable with small doses in the surviving blood vessels perfused with Tyrode's solution than in the living animal. In perfusion experiments more powerful and constant effects are obtained with small quantities of this dye than by the use of any other vasoconstrictor. The dye acts directly upon the muscle, and not through the sympathetic nerve supply. The reaction seems to be of a tachyphy-lactic nature. It has, as yet, been found impossible to demonstrate any effect of the dye upon strips or rings of arteries suspended in Dale's or Tyrode's solution, but there is some evidence that the dye must be reduced before the characteristic reaction occurs.

The relation between tension and speed of shortening in human muscles by a new method. W. O. FENN.

The subject sits with one leg hanging over the edge of a table. He tries to extend his leg against an isometric lever which records the tension developed. The leg is suddenly released by pulling a pin and a pointer records on a drum the rate with which the leg moves forward. From this tracing the acceleration of the leg is determined. Since the muscle tension is proportional to the acceleration the observations give information concerning the rate with which the muscle tension decreases as the speed of movement increases. It is found that the tension decreases 3.1 per cent for an increase in the rate of shortening of the muscle of 10 per cent of its length per second. A similar figure calculated from the experiments of Hill and those of Hansen and Lindhard gave the values 7.3 per cent, 5.9 per cent and 8.0 per cent for three different subjects. It is believed that the higher figures are complicated by changes in the innervation of the muscles. Assuming that the smaller figure of 3.1 per cent can be extrapolated to higher speeds it is concluded that a sprinter could exert no external tension at maximum speed of leg movement.

Further studies on the mechanism of ovulation. MAURICE H. FRIEDMAN.

In a recent paper Hill and Parkes (Proc. Royal Soc. 1930, cvii 30,) voice the opinion that the formation of a corpus luteum is the direct result of the act of ovulation itself, and "is not connected with the subsequent release from the anterior pituitary body of the stimulus to luteinisation." In support of such hypothesis the authors recall the experiments of O'Donoghue, and of Ancel and Bouin, in which the artificial rupture of follicles in the rabbit is said to have led to the formation of corpora lutea.

To test this hypothesis the experiments of O'Donoghue were repeated and extended.

Through a flank incision under ether the right ovary of several rabbits was exposed and all the large follicles carefully punctured. After a varying period of time the ovaries were removed for examination. For controls against the factor of operative trauma, follicular puncture was performed

on one ovary in each of several rabbits at varying intervals after coitus (five minutes to three hours). From such experiments it was found that

1. Follicular puncture in rabbits known to be in heat does not lead to the formation of corpora lutea.

2. Follicles punctured in rabbits very shortly after coitus develop into normal corpora lutea indistinguishable from the corpora lutea in the ovary on the unoperated side.

In further experiments it was found possible to produce corpora lutea in one ovary without affecting the ripe follicles in the contralateral ovary. This is achieved by the injection of a small quantity of an extract of urine of pregnancy directly into the follicles of one ovary. The corpora lutea so produced, however, are by no means entirely normal structures. Just how far these artificial corpora lutea deviate from the normal is at present under investigation.

Fatigue and recovery in muscle. E. GELLHORN.

A. Fatigue. 1. The fatigue of the sartorius muscle of frog, which is stimulated by condenser discharges, can be delayed by an excess of calcium chloride in Ringer's solution, although this solution does not preserve irritability of resting muscle as well as Ringer's solution. Therefore, the quantitative composition of the optimal salt solution depends upon the permeability of the cell.

2. The calcium effect of fatigue is a specific one. Neither Sr nor Ba nor Mg can replace it. But these kations can delay the fatigue although to a lesser degree than calcium when added to Ringer's solution containing $8.1 \text{ M} \times 10^{-4} \text{ CaCl}_2$. The cause of this specificity is discussed.

B. Recovery contracture. If the sartorius of biceps of *Rana esculenta* is stimulated with condenser discharges in periods of 5 minutes with a frequency varying between 45 and 150 per minute, a contracture is brought about in the beginning of the second and each following stimulation periods, provided the muscle is allowed to recover in an aerated salt solution. Wide changes in the K and Ca concentration of the Ringer's solution are without influence. The contracture also occurs when isotonic solutions of Na or Li salts are employed. The phenomenon is suppressed by replacing the salt by a non-electrolyte. In favor of the assumption that the contracture is a recovery contracture are the following observations: 1. The phenomenon does not occur if the frequency is too low to produce even a slight fatigue. 2. It decreases from the second to fourth stimulation period, i.e., with increasing fatigue. 3. The changes in the shape of the contractions (Funke's nose) during the recovery contracture show a temporal sequence just the opposite of that in fatigue.

It is assumed that changes in the surface layer of the muscle cell bring about the recovery contracture. In favor of this hypothesis are the facts that: 1. The recovery contracture is increased with increasing duration of the electrical discharges which were used to stimulate the muscle. 2. Non-electrolytes which suppress the phenomenon bring about changes in the surface layer of cells.

The respiratory quotient of the recovery period following strenuous muscular exercise. C. L. GEMMILL.

The respiratory quotient of the recovery period following strenuous muscular exercise was obtained by a subject running sixty-five yards in ten

seconds on a tread mill and making a continuous collection of the expired air for three hours after the run. The subject was in a basal condition before the run. The oxygen consumption returned to the base line within two hours after the run but the carbon dioxide output showed rhythmic variations above and below the base line, these variations only coming within normal limits towards the end of the three hour period. Before doing the work experiments several basal determinations were made over the same period of time to obtain the normal variations and to accustom the subject to breathing through a mouth piece for three hours.

With oxygen debts of 5.25, 5.52, 7.78 liters of oxygen the "excess" respiratory quotients of the three hour recovery period in three experiments on the same individual were 0.85, 0.86, 1.10, within the same range as obtained in moderate work. If the "excess" respiratory quotient is calculated at the end of one hour with an incomplete recovery period, higher values were obtained, 1.14, 1.46, 1.31.

A modified Warburg apparatus and some applications. R. W. GERARD.

The usual form of Warburg apparatus has the manometer limbs outside of the thermostat and each manometer shaking to and fro about a horizontal axis, at which is placed the screw for adjusting the fluid levels. With small chambers it becomes desirable to have the entire manometer (or all the gas space) immersed in the thermostat, and a further considerable advantage is obtained if readings can be made without disturbing the regular shaking. The manometer mounting and shaking devices have been modified to this end. The adjusting screw passes vertically between the manometer limbs and again forms the axis for the shaking movement. The chamber thus describes a considerable arc, while the limbs have a very small forward and backward movement and can be read in motion through a glass wall of the thermostat. The manometer is mounted in the bath, making connection with the shaking system above. The bath also contains an empty 10-liter bottle, connected by metal and short rubber connections with the "open" limb of each manometer, thus eliminating barometric effects (Frapp).

The manometric technique has proved applicable to the determination or preparation of gas mixtures of low oxygen tension. Thus a manometer may be filled with pure N_2 or H_2 at atmospheric pressure and, after it has reached equilibrium in the bath and the fluid level has been noted, a rapid movement of the stop-cock serves to admit air. The original volume is now restored by adjusting the manometer fluid level and the increased pressure obtained. This, corrected for water vapor, divided by atmospheric pressure and multiplied by 100:5 gives the percent of oxygen. Similarly, a chamber containing alkali in the bottom and a substance oxidizing in alkali (as pyrogallol) in the side arm can be filled with N_2 , H_2 , CO, etc. containing less than 3 percent O_2 , brought to equilibrium and tipped. The pressure decrease gives in simple fashion the oxygen percentage. Analogous arrangements, with the chambers filled with hydrogen, containing a reducible substance in the bottom and a hydrogenation catalyst in the side arm, to be tipped in after equilibrium is attained, permit the determination of hydrogen numbers and the like. Adsorption of hydrogen by the catalyst must be allowed for.

The influence of methylene blue and of sodium iodoacetate on glycolysis. R.

W. GERARD.

Since methylene blue can be reduced by nerve, thus furthering oxidations when oxygen is absent or rendered ineffective by cyanide, it seemed possible that it would decrease anaerobic glycolysis. This was studied by Warburg's method, depending on the manometric measurements of CO_2 liberated from bicarbonate by a stronger acid formed in the tissue. The glycolysis (anaerobic acid formation) of frog nerve at 21°C . was, however, increased 50 per cent or more by the addition of 0.05 per cent methylene blue. Similar experiments with frog muscle demonstrated a very great action of the dye, glycolysis being increased on the average about 10 times. This high rate falls fairly rapidly with time, but even after five hours is well above the control. The magnitude of the effect increases with the dye concentration, at least up to 0.07 per cent, and there is hardly any rise obtained with a concentration of 0.0025 per cent. The fall in glycolysis rate with time after adding the dye might depend on its progressive reduction to the leuco form, but since high glycolysis may persist after all color has disappeared, it is not certain that the leucobase is inactive. Adding a second portion of M.B. evokes a further increase. Respiration is also greatly increased by the dye, probably secondarily. The dye effect on chopped muscle is less than on intact. Mouse muscle at 37° or 21° is much less affected.

Frog and mouse liver and brain glycolysis are also increased up to 100 per cent by M.B. Mouse sarcoma glycolysis is hardly affected by M.B. (increases up to 25 per cent) at 21° or 37°C .

M/15 sodium iodoacetate cuts the glycolysis of muscle in the presence of methylene blue to roughly one-fourth. In equal molarity, NaF is about half as effective and NaI without action. Similar relations hold for nerve. In the absence of methylene blue, the manometer readings are reversed by iodoacetate, as if acid is being removed by the muscle rather than formed. None of these glycolysis inhibitors depress respiration except in greater concentration. A depression of sarcoma glycolysis similar to but definitely more than in the case of muscle follows the addition of iodoacetate, a more moderate depression the addition of fluoride. Aerobic and anaerobic glycolysis are both affected.

Sodium iodoacetate injected into mice intraperitoneally every two days in sublethal doses does not prevent or influence the growth of implanted sarcoma.

Creatine in nerve and muscle. R. W. GERARD and N. TUPIKOW.

We have previously (Proc. Soc. Exp. Biol. and Med., 1930, xxvii, 360) demonstrated the existence of creatine in nerve in three fractions: one behaving as "free" creatine (F), a second "bound" as phosphocreatine (B), and a "residual" fraction, not extracted under our experimental conditions (R). The analytical methods have been carefully checked and duplicate determinations agree within 5 per cent. In frog nerve in O_2 , the total creatine is about 160 mgm. per cent, of which almost exactly $\frac{2}{3}$ is F, $\frac{1}{3}$ B and $\frac{1}{3}$ R. For muscle with 500 mgm. per cent total creatine, 46 per cent is F, 47 per cent B and 7 per cent R. Prolonged anoxia causes half the bound creatine of nerve and $\frac{2}{3}$ that of muscle to become free. The change is nearly maximal in 1 hour in nerve.

In 12 parallel experiments with nerves kept for 2 hours in O_2 at rest or

stimulated, the latter group showed an average breakdown of 5 mgm. per cent of B to F, as compared to the controls. The decrease of bound and increase of free creatine of the stimulated as against the resting nerves was clearly present in 11 of the 12 individual experiments. This breakdown of phosphocreatine during activity is in harmony with the similar conclusion reached by Gerard and Wallen (This Journal, 1929, lxxxix, 108) from a study of nerve phosphates, the changes in bound creatine and phosphate agreeing.

Ten sets of experiments on the influence of CO₂ on the creatine distribution in nerve showed likewise a consistent breakdown of B to F. With 100 per cent CO₂ the maximal change is present after 5 to 10 minutes' exposure; with 5 per cent CO₂ in O₂, experiments were continued for 1 to 2 hours, though maximal effects may have been reached earlier. Average values obtained as per cent of total were: F—in O₂, 41, in CO₂, 47, in O₂ plus 5 per cent CO₂, 43; B—in O₂, 40 in CO₂ 33, in O₂ plus 5 per cent CO₂ 38; R—in O₂, 19, in CO₂, 20, in O₂ plus 5 per cent CO₂, 19. In muscle over half the bound creatine became free when exposed to CO₂, and half of that broken down was again reformed during a subsequent half-hour in O₂.

H₂S led to a marked breakdown of B in muscle and nerve (13 experiments) with no recovery in O₂ over several hours. R increases slightly at the expense of F during this time. The breakdown in H₂S is maximal at 15 minutes' exposure and almost $\frac{3}{4}$ of the bound creatine of muscle and half that of nerve is freed—as in the case of prolonged anoxia. CN likewise, applied as a gas or as NaCN in solution, frees a third or more of B of nerve in 20 minutes. A 10 per cent increase of R was also noted. Carbon monoxide containing 2 per cent O₂ also leads to the same changes, a loss of B which appears largely in F but partly in R. The action appears to be less rapid than in the case of the other respiration inhibitors, and is largely reversible in O₂ or by light. In nerve, however, the residual fraction remains high even after recovery, in muscle apparently not. In nerve, $\frac{1}{4}$, in muscle, $\frac{1}{2}$, of B is broken down in 1 to 2 hours, and half of this amount is reformed in O₂ or by light. The breakdown of B in nerve in CO is increased by tetanization.

Methylene blue or cresyl blue in 0.1 per cent isosmotic solutions at pH 7.2 leads to a loss of half the bound creatine present in muscle, even when this is independently reduced to a third by anoxia or chopping. In nerve, the dyes have no effect in O₂ or N₂ but slightly retard the breakdown in cyanide (14 expts.).

Sodium iodoacetate, M/500, increases the loss of B in N₂, M/200 does so in N₂ or O₂.

The cyanide insensitivity of paramecium. R. W. GERARD and L. H. HYMAN.

Paramecium caudatum was grown in pure culture, washed twice by centrifugation from a calcium-containing fresh water and a moderate suspension made in N/150 phosphate buffer at pH 7.4 or 8.0. Six-tenths to 2.0 cc. quantities were introduced into Warburg manometers for the determination of oxygen consumption. NaCN was added to some suspensions in N/40 to N/4000 concentration at the start of a run or after a control period. In some cases it was tipped from a side arm, more often the manometer opened and the cyanide introduced from outside. In most experiments a fresh N/1 cyanide solution was almost neutralized to litmus, prop-

erly diluted in buffer and rapidly placed in the vessels. Experiments were run by the usual method with alkali to absorb the carbon dioxide formed, and also by the differential fluid volume method not requiring alkali.

Respiration in the buffer solution alone is depressed to about half that in water. (The possibility of a trace of pyrophosphate present in the phosphate was not excluded.) In twelve experiments at pH 7.4, the addition of *neutralized* cyanide to a concentration of N/100 to N/200 had no further effect than the buffer alone. N/40 cyanide led to about 50 per cent inhibition. NaCN added without previous neutralization to the buffer at pH 8 often, but not always, decreased respiration, the decrease being associated with death of the protozoa. Thus: with N/800 CN, up to 30 per cent inhibition was obtained; with N/4000, hardly 10 per cent; with N/200, values varying from no to complete stoppage of respiration. We conclude that with proper control of alkalinity in phosphate buffer at pH 7.4 the respiration of paramecium is essentially cyanide stable, as claimed by Lund (This Journal, 1918, xlv, 365). Even in the absence of buffer, only minimal depressions (to 15-20 per cent) are obtained with neutralized cyanide (N/100). Shoup (personal communication) has obtained similar results.

A contribution to the study of the osmotic relations between blood and gastric juice. ALFRED GILMAN and GEORGE R. COWGILL.

Pure gastric juice was obtained subsequent to histamine stimulation from two dogs, one possessing a Heidenhain, the other a Pavlov pouch. Coincident with the collection of the juice a blood sample was drawn. The osmotic pressure of the gastric juice was considered to be a close function of the total Cl content; the fixed base concentration of the blood was taken as the index of the osmotic pressure of this fluid. Changes in the fixed base of the blood were produced by alternate hydration and dehydration of the experimental animal, and by this procedure values ranging from 154-180 MEq were obtained. Concomitant with these changes in the blood, parallel variations in the total chloride level of the gastric juice were observed (160-184 MEq). Both experimental animals gave almost identical results. The authors consider these data to support the view that the concentration of Cl in the gastric juice is regulated chiefly by the total ionic concentration of the blood, the two solutions being in osmotic equilibrium. This work is preliminary to more extensive studies involving actual measurements of the osmotic pressure of these fluids.

Temporal summation of subliminal visual stimuli. RAGNAR GRANIT.

A flash of light lasting for 0.0113 sec. is adjusted to a strength just below the absolute threshold of vision. When this flash is followed by a second subliminal of still shorter duration, it is found that the second flash sums with the after-effect of the first subliminal to give a visible effect. The shorter the interval between the flashes, the smaller the quantity (intensity \times duration of flash) with which the second subliminal is capable of raising the after-effect of the first to threshold value. By measuring this quantity at various intervals between the flashes, it has been possible to determine the level of the subliminal excitatory state as a function of time.

A full account of the results will be published by Mr. W. A. Davis and the author in this Journal.

The influence of adrenalin on the coronary flow in the dog. C. W. GREENE and JAMES A. ATKINS.

The reaction of the coronary arteries of the dog to adrenalin has been tested by the method of injecting solutions of this hormone into the jugular vein and measuring the effects in terms of variation in outflow from the coronary sinus. The rate of coronary flow was recorded graphically by means of a water (blood) manometer along with blood pressure by the Ludvig manometer. The blood pressure was stabilized as far as possible by an overflow reservoir at an elevation of 100 or 80 cm. The blood was kept fluid by heparin and the coronary blood was returned at a constant rate by a Woodyatt pump.

After a latency of 9 to 11 seconds the flow from the coronary sinus rapidly and actively increased to a maximal in 20 to 40 seconds, then slowly decreased, according to the sensitivity of the animal, through 100 to 180 seconds, or even more. Of 52 experiments on 12 dogs, 61 per cent were typical coronary dilatations, 29 increasing 100 per cent or more.

The great rise in arterial blood pressure passively augments coronary flow, but when this is largely equalized the active factor in dilatation is clearly shown. In like manner the effect of the great augmentation of heart rate can be ruled out. Without the pressure equalizer the active dilatation is still clear. As an example, in dog 63, test 10, 0.1 cc. of 1-1000 adrenalin, blood pressure response increased by 117 per cent, the coronary dilatation by 170 per cent, after 60 seconds the blood pressure was just under normal but the coronary dilatation was still 40 per cent. There is no apparent causal relation of change in coronary flow to the change in heart rate. Furthermore, the increase in coronary flow may occur with no change in the other two factors or with change in the reverse direction. All these confirm the view that adrenalin causes an active coronary dilatation in the dog to which must be added the passive dilatation from the associated rise of blood pressure.

An analysis of the efferent pathways and vasomotor control of the coronary circulation in the dog. C. W. GREENE.

Porter's first demonstration of coronary vasoconstrictor fibers in the vagus pathway has been confirmed by Maas, Wiggers, Morawitz and Zahn, Anrep and co-workers, and by the writer. The presence of coronary dilators, discovered in the cardiac nerve trunks from the stellate by Maas, has also been abundantly confirmed. These coronary dilator efferent paths are extended by the present series of experiments to the filaments from as low as the 6th ganglion of the thoracic chain. The vasodilatation by these lower paths is greatest in amount in the filaments arising from the 5th and 6th thoracic ganglia on the left side. The coronary vasodilators run in the same trunks as the cardiac accelerators, but not necessarily in corresponding proportions nor always present. The extremely great variation in amount of both vasoconstrictor and vasodilator control in different animals is strongly emphasized.

Glycogen formation from amino acids. ESTHER M. GREISHEIMER and F. P. ARNY.

Rats were starved for a period of 16 to 20 hours, then fed the amino acid to be investigated, and killed six hours later. Liver glycogen and lipid were determined. The amount fed to each rat was about 2 grams, suspended in distilled water. Forced feeding was performed.

Glycine appeared to make the animals ill. The amount of glycogen in the liver was 0.28 per cent, the lipid 5.38 per cent.

d-l Alanine apparently produced no ill effects. The glycogen content averaged 1.21 per cent, the lipid 6.58 per cent.

d-Glutamic acid proved rather irritating. The glycogen content averaged 0.31 per cent, the lipid 8.12 per cent.

Since earlier studies showed the fasting glycogen content to be 0.32 per cent, it seems that neither glycine nor glutamic acid led to the formation of glycogen under the above experimental conditions. Alanine, however, was able to produce glycogen.

The differential calorogenic action of the liver and adrenals. FRED R. GRIFFITH, JR. and F. E. EMERY.

If the liver contributes to the usual calorogenic response following an injection of adrenalin or stimulation of the adrenal glands, it might be supposed to do so as a result of the stimulation of its sympathetic nerve endings and the same response might be expected, unobscured by any other concomitant of the adrenalin reaction, from direct stimulation of its nerves alone. Stimulation of the hepatic nerves does produce a 10-12 per cent increase in oxygen consumption; this increase is quite transient, being over in 5 to 10 minutes in spite of continued stimulation. If one adrenal is now stimulated through its splanchnic nerve the usual calorogenic response of adrenalin follows; i.e., previous "exhaustion" of the liver does not impair the response to adrenin. If, on the other hand, the adrenal is stimulated first, with the usual calorogenic response, immediate stimulation of the hepatic nerves produces the usual, transient, hepatic increase in metabolism; i.e., the adrenalemia does not seem to have "exhausted" the liver in the same way as does stimulation of its nerves. Or, in other words, the liver does not seem to be involved in the calorogenic response resulting from stimulation of the adrenal gland.

Amino acids as factors in growth and differentiation (initial experiments).

F. GUDERNATSCH and O. HOFFMAN.

An attempt has been made to determine the effect of isolated amino acids on growth and differentiation of the amphibians, *Rana sylvatica* and *pipiens*. The entire diet given, including the basal diet, was made up of chemically pure substances. The only nitrogen-containing substances fed to the animals were the amino acids. Fourteen of the naturally occurring acids were used in solution (except cystine) in varying concentrations. They were given singly, in all possible combinations of two acids, three acids and an arbitrary progressive series of mixtures containing from three to fourteen of the acids.

The method of attack resulted in data which can be classified under the four groups: 1, life span of the various groups; 2, rate and extent of growth; 3, appearance of somatic features which indicate differentiation; 4, distribution of mortality. In many cases animals were maintained for a long time without progressive differentiation. In leucine neither growth in size nor rate of growth was great, yet maintenance was unusually good. Phenyl-alanine resulted in good growth and the percentage of animals showing differentiation was high. Tryptophane, while not producing good growth, was an exceptionally good differentiating factor. In dif-

ferentiation, tyrosine was one of the most outstanding—distinct evidences being produced earlier and in more animals than in other groups. Cystine proved one of the most favorable influences on growth. In cystine plus tryptophane differentiation began very early comparatively, and maintenance was among the best of the series. Also, arginine is a more favorable growth than differentiation factor.

Optimal results were obtained in the mixtures containing the larger number of amino acids. In general, the only signs of differentiation were the appearance of hind limbs and, in a few instances, of fore limbs and tail resorption. The amino acids which facilitated growth delayed the differentiating influence of di-iodotyrosine.

Cardiac output, mean circulation time, total blood volume and heart-lung blood volume under physiological and pathological conditions. W. F.

HAMILTON, J. W. MOORE and J. M. KINSMAN.

Various conditions which result in an increase in stroke volume of normal men and animals cause also a marked and quantitatively measurable increase in the intrathoracic blood volume. This is brought about through the increased left ventricular filling pressure acting on the elastic lung capillaries.

If the left ventricle is weakened through disease (congestive failure) an increased filling pressure is necessary to get a more or less adequate stroke volume (Starling's law of the heart). This increase in left ventricular filling pressure results in a marked and quantitatively measurable engorgement of the intrathoracic blood spaces. The stroke volume and the intrathoracic blood may either one approach the normal but the relation between them is strikingly different from the normal.

On recovery from decompensation the greatest and most constant change is a movement toward normal of the ratio between stroke volume and heart-lung blood volume. The stroke volume, the minute volume and the heart-lung blood volume may increase, decrease or remain unchanged.

The mean circulation time,—the time from the middle of the injection period to the center of gravity of the time concentration curve—is always increased in congestive heart failure, returning toward normal on compensation.

The total blood volume (vital red method) is almost uniformly increased by twenty per cent or more in congestive heart failure.

These considerations are illustrated by spot diagrams embodying the results of 100 experiments on man and 50 experiments on the dog.

Mechanism of postural changes in vital capacity in relation to cardiac dyspnea and the storage of blood in the lungs for emergencies. W. F. HAMILTON and A. B. MORGAN.

The well known reduction in vital capacity on recumbency is accompanied by an equal reduction in total capacity, since, contrary to earlier observers, the residual air is shown to remain unchanged.

X-ray and mechanical measurements indicate that this reduction in total capacity on lying down is accompanied by no decrease, and usually by an increase in the volume of the thoracic cavity in both the completely expired and completely inspired position.

It would seem from the above that the lungs serve as storage for blood which is poured out of the dependent veins when one lies down. This

blood storage function of the lungs encroaches upon their respiratory function and, in excess, accounts for the reduced vital capacity and dyspnea of cardiac disease. In the normal man it may act as an emergency reserve.

It can easily be proven that the postural change in vital capacity is due to increased return of blood to the chest which is not pumped out again because of the low systemic demand. If blood is trapped in the four extremities by cuffs around the thighs and upper arms, blown up to the diastolic level, the recumbent vital capacity is increased so that it may equal or exceed the vital capacity in the standing posture.

The energy cost of the "push-up" exercise. H. E. HAMLIN.

The object of these tests is to determine the energy cost of individual athletic exercises. In this report are summarized the results of twenty-five determinations on twenty-one men and four women students for the exercise known as the "push-up." Twenty of them were students in an elective course in the physiology of exercise, and the remainder were all interested in the investigation. It was a varied but ideal group on which to run such a test. The average weight of the men was 68.2 kgm. and that of the women 55½ kgm.

The "push-up" is a fairly strenuous exercise, for which reason each student was asked to raise himself only ten times during the test and at a moderate speed. It is mainly an exercise of the extensor muscles of the body. The average time for the ten push-ups was 0.33 min., the rate being set by a metronome. All tests were made in the post-absorptive state.

The Collins Chain Compensated gasometers and Haldane gas analyzers were used to determine the respiratory exchange.

The energy cost for each student was measured under the following conditions: For the basal condition, the starting position before the exercise, and during the exercise and its recovery. Approximately a ten minute period was allowed for recovery, after which a check recovery of six minutes was run on most of the subjects.

The average pulmonary ventilation in litres per minute for basal conditions was about 5, and the percentage increase during exercise and recovery was 92 per cent.

The oxygen consumption for ten minutes under basal conditions averaged 2.55 litres, and was increased 53½ per cent during the exercise and recovery.

The average carbon dioxide elimination for the ten minute basal test was 2.15 litres, and this was increased 79½ per cent during exercise and recovery.

The average "gross" cost in energy for the ten push-ups was 7.06 Cal. The average "net" cost from the starting position was 5.874 Cal. Reducing the "gross" cost to the energy cost per push-up per kgm. of body weight shows that the average cost on this standard basis is 0.0108 Cal. The extremes were 0.0070 and 0.0188 Cal. Thirteen of the twenty-five students had an energy cost of between 0.0070 and 0.0102. Four with high energy costs had great difficulty in completing the exercise.

Many of the results show that there may be a correlation between the energy cost and the body weight, but others show that some other factors may be involved.

The vital hormone of the adrenal cortex. FRANK A. HARTMAN and KATHARINE A. BROWNELL.

A concentrated extract containing the vital hormone of the adrenal cortex can be made by the very simple process of extraction with ethyl ether and ethyl alcohol. This extract is not toxic and contains but a small amount of epinephrin.

Completely adrenalectomized cats can be kept alive in good condition by the injection of such an extract. One cat was kept alive for nearly nine months, dying because the extract was diminished. The adrenals need not be removed in two stages to insure survival. If an adequate amount of extract is given through the critical stage cats with both adrenals removed at one operation do as well as those operated upon in two stages. Adequately treated adrenalectomized animals behave in every way like normals.

Young animals grow to maturity and successfully raise litters (rats). The vital hormone enables adrenalectomized rats to resist cold almost as well as normals. Normal rats, cortin treated adrenalectomized rats, and adrenalectomized rats injected with an equal volume of salt solution were exposed to a temperature of 10°C. for ten hours. The average temperature fall was as follows: normals, 38.6 to 36.88°C.; cortin treated, 37.76 to 35.33°C.; NaCl treated, 37.24 to 27.28°C.

The cortical hormone raises the resistance of adrenalectomized rats to bacterial toxins. A majority (71 per cent) of cortical treated rats survived the injection of a dose of typhoid vaccine which killed all of the controls. Likewise we have been able to show an increase in the resistance to chronic intoxication from bacterial toxins.

Cats allowed to pass into the last stages of adrenal insufficiency which just precede death have been revived by cortical extract.

A subject of Addison's disease with notably dubious prognosis was revived and has been kept alive for eight months by injection of this extract.

Two cases of toxic goiter have shown improvement with similar treatment.

Two cases of muscular atrophy have been benefitted by cortical extract. Fibrillation of skeletal muscle has disappeared and the general condition has much improved.

Cortical extract increases the threshold for fatigue in the asthenia not only of Addison's disease and toxic goiter but in some other conditions. In one patient, the amount of work (by ergograph) which could be accomplished without fatigue was ten times greater after a few days' treatment than it was before.

Stabilization of the fundamental rhythm of the alligator heart. E. NEWTON HARVEY.

With what constancy can an excised heart beat? By means of the Loomis chronograph (see Loomis and Harvey, *Science*, 1929, lxx, 559), a device for automatically recording rates of rhythmic processes over long time periods, a study has been made of the rate of beat of the alligator heart excised from the body and kept at $25.2 \pm 0.02^\circ\text{C}$. in Ringer's solution + 0.1 per cent glucose, saturated with oxygen. Turtle's hearts will beat for 24 to 36 hours under these circumstances with only a gradual slowing; they show great variation in behavior, some beating with only a 2 per cent change in rate over a 5 hour period, and others exhibiting large regular rhythmic changes

in rate (Loomis, Harvey and MacRae, Journ. Gen. Physiol., 1930, xiv, 105). None of the alligator hearts are capable of maintaining even a fairly constant rate in oxygenated Ringer + glucose alone, but continually slow down, often to one-half the original rate in 20 minutes. Their beat can be stabilized by adrenalin or ephedrin, one part per million being a good concentration. The adrenalin effect wears off but the ephedrin effect may last for hours and so stabilize the beat that the variation in rate is only 0.58 per cent (from 40 beats per minute) over a one hour period. The difference in behavior of turtle and alligator may be connected with a greater normal concentration of adrenalin in turtle's blood. The minimum detectable concentration of adrenalin is 10^{-7} and ephedrin is 10^{-7} grams per cc. Sudden changes in rate are very characteristic of the alligator heart but these disappear after stabilization, when occasional slight rhythmic changes in rate only are to be noted.

Studies on the possibility of gluconeogenesis from fat. ESTELLE E. HAWLEY and JOHN R. MURLIN.

1. *Phlorhizinized dogs.* Wertheimer reports (1926) that sugar is formed in the phlorhizinized dog from the fat stores especially under the influence of insulin. Such an animal therefore is less sensitive to insulin than the normal, and his recovery from insulin shock is more rapid. The fatty liver is alleged to transform fat into carbohydrate and so prevent extreme hypoglycemia or to replace the lost sugar more rapidly than it could be replaced from the glycogen stores. Also that the phlorhizinized dog reacts more quickly to adrenalin than does the normal dog.

The experiment has been repeated in this laboratory—the data including R. Q. experiments, D:N, blood sugars and blood fats.

In the dogs so far studied, we have been unable to confirm Wertheimer's experiments. In each case convulsions appeared more quickly in the phlorhizinized than in the non-phlorhizinized dog, and the recovery was not so rapid. Sugar was necessary to save one dog. One dog died in coma and another dog reacted so quickly to the insulin that the experiment had to be discontinued.

The D:N ratios and R.Q.s do not indicate gluconeogenesis from fat in these experiments.

2. *Human subjects.* The significance of very low respiratory quotients which have been reported after high fat feeding is not yet clear. In our work several human subjects have subsisted on 40 per cent cream for several days, after which, following a heavy meal of such cream, R.Q.s as low as 0.63 and 0.65 several times have been obtained both by the Tissot-Haldane and the Benedict Universal closed-circuit methods. The total fat, acetone bodies, sugar and CO_2 combining power of the blood and acetone bodies and ammonia of the urine have been followed. The corrections possible from these data do not suffice to raise the R.Q.s to the level of fat combustion. The conversion of all the glycerol to glucose would not affect the R.Q. materially. It is possible, therefore, that these low quotients signify gluconeogenesis from fat and storage of the resulting sugar temporarily as glycogen.

Analysis of potential of Limulus medial cardiac nerve. PETER HEINBECKER.

At the physiological meetings 1930 the writer reported the separability of fiber groups in the vagus nerve of the turtle which are responsible for

initial inotropic and chronotropic changes respectively in the auricular beat. Search for a possible explanation of the underlying mechanism led to studies on the medial cardiac nerve of *Limulus*. Using the cathode ray oscillograph as a recording device, the results obtained show that there occur series of automatic discharges from the ganglion cells of this nerve at a rate comparable to that of a normal heart. Each of these discharge series begins with scattered volleys of low potential which increase in frequency, number and amplitude up to a maximum. The discharges are periodic, last for a second or more, and are followed by a similar period of quiescence.

By analysis of records of electrically stimulated and of automatically developed responses it seems possible to separate the discharges into two types. The first potential complex arises in fibers which extend more or less completely for the length of the nerve cord. It has a definite threshold, conduction rate and absolutely refractory period. The potential is propagated in both directions along the nerve without apparent delay. Just threshold responses of this complex may not elicit responses of the second type, but well developed first complexes stimulated at 80 to 100 times per minute elicit volley discharges of the second type, one series corresponding to each stimulus. At stimulation frequencies greater than 100 per minute the second type of response takes up its own independent rhythm approximating in time period that of the normal heart beat, as if synapses of relatively long refractory period were showing Wedensky inhibition. Strong stimulation of the first complex increases the amplitude of the potentials composing the second type of responses. The potentials of the second order do not arise in fibers which extend the whole length of the nerve cord but arise at various levels.

Apparently the first complex could control the intrinsic rhythm of the heart, and the second complex produce the immediate stimulation of the heart muscle cells.

Histological studies of Patten would seem to permit the correlation of the first potential complex with the large fibers and their giant bipolar ganglion cells. The second type of complexes could then arise from the fibers associated with the smaller multipolar ganglion cells found chiefly in the outer layer of the nerve cord.

Studies on intestinal obstruction. RAYMOND C. HERRIN and WALTER J. MEEK.

In a series of dogs three different types of intestinal fistulae were made aseptically in the upper jejunum and the lower ileum. The Thiry, Thiry-Vella and a modified Thiry fistula in which the intra-abdominal end was patent to the lumen of the intestinal tract, made it possible to insert into the isolated loop a rubber balloon which could be inflated with a pressure of 80 to 90 mm. of Hg. In the first type of fistula the intestinal juice secreted by the loop was prevented from accumulating by means of a drainage tube; in the second the secretion passed readily through the second opening to the outside of the body; and in the third it returned to the intestinal tract where it could be absorbed.

Within 7-14 days after inflation the blood chlorides decreased from a normal range of 425-530 mgm. per cent to 210-325, non-protein nitrogen rose from 23-32 mgm. per cent to 65 or more, and blood volume, indicated by hemoglobin, red cell counts and actual volume determinations, showed a

reduction of 27 to 40 per cent. Analyses of skin and muscle showed marked decreases in salt and water content. The alkali reserve remained constant or fell slightly unless vomiting had occurred in which case it increased. After a few days' distention the animals refused food and water and this hastened a fatal outcome. The symptoms and blood findings it will be noted are the same as those described for uncomplicated intestinal obstruction and this leads us to believe that in our experiments the same factors are operating.

There was no difference in the response of the dogs with the Thiry and Thiry-Vella fistulae, but those with the modified Thiry required a longer period before the typical blood changes appeared. Intravenous injections of Ringer's solution gave only temporary improvement, dehydration soon re-occurring.

Water balances were made on several animals. Some of the dogs vomited but many did not. Urine secretion was regularly decreased. The greatest losses in nearly all cases were due to the insensible perspiration and the secretions from the fistulae. As much as 550 cc. have escaped from a Thiry fistula 20 cm. long in 24 hours. Distention is a strong stimulus to intestinal secretion and in obstruction this must work in a vicious circle. In view of the greatly reduced intake and the large output by way of the fistulae the dehydration and decreased blood volume are easily understood.

Dogs with fistulae low down in the ileum were very resistant and showed little change in the blood picture.

Our experiments are believed to support the view that death in uncomplicated intestinal obstruction is due neither to a bacterial toxemia from the intestinal contents nor to the absence of any special substances in the gasteroduodenal secretions, but to a loss of salt and water.

The effect of emotional stress on blood fat. HAROLD E. HIMWICH and JOHN F. FULTON.

Cannon has shown that violent emotional outbursts are associated with an increased secretion of adrenaline. Since it has recently been demonstrated in this laboratory¹ that injections of adrenaline raise the level of blood fat, it seemed to us desirable to study blood fat in emotional states. Fifteen observations have been made on cats and 3 on dogs. Two cats were hemidecerebrate, and from three both cerebral hemispheres had been removed two months previously. Blood samples were drawn before and after each normal cat had been exposed to a barking dog for 15 minutes. The thalamic preparations were excited by pinching their tails for several minutes. The blood samples were analyzed for fat, using our modification of the method of Stewart and White and for sugar by the technique of Hagedorn and Jensen.

The results were strikingly uniform. The concentration of fat and sugar increased in every observation. In one normal cat the increase in fat was as much as 250 per cent above the resting value. In the thalamic preparations the increase amounted to as much as 75 per cent, even in an emaciated animal. The work of Wilenko,² Cori and Cori,³ and Colwell and Bright⁴

¹ Himwich, H. E. and M. L. Petermann. *Proc. Soc. Exper. Biol. and Med.*, 1930, xxvii, 814.

² Wilenko, G. *Biochem. Zeitschr.*, 1912, xxi, 44.

³ Cori, C. and G. Cori. *Journ. Biol. Chem.*, 1928, lxxix, 309, 343.

⁴ Colwell, A. R. and E. M. Bright. *This Journal*, 1930, xcii, 543.

has demonstrated that injections of adrenaline diminish the oxidation of carbohydrate; therefore, despite the rise of the level of blood sugar, it does not seem likely that carbohydrate is the preferred foodstuff under violent emotional stress (anger and fear), but rather that the increased concentration of blood fat is a result of the mobilization of fat depots in response to the call of the tissues for fuel. These observations also suggest that in a highly emotional state there is a shift in the foodstuffs utilized, *i.e.*, more fats and less carbohydrates are used to supply the energy necessary for the exigencies of the moment. The fact that insulin diminishes the level of blood fat, particularly when artificially raised by adrenaline, is in accord with this conception. We are now undertaking studies of the respiratory metabolism during emotion.

Respiratory exchanges during work and recovery. F. A. HITCHCOCK.

Experiments have been carried out in which the respiratory exchanges were measured by the Tissot method 1, during a period which the subject spent in sitting still on the bicycle ergometer, 2, during a ten-minute period of work on the ergometer the rate of work varying from 0.65 to 1.9 Calories per minute, and 3, during a recovery period of about twenty minutes spent in sitting still on the ergometer. This recovery period was divided into two or three parts. With the smaller amounts of work the R. Q. during work was approximately the same as during rest. In the recovery period the oxygen consumption returned to normal more rapidly than the carbon dioxide production. This resulted in a sharp rise in the R.Q. With greater amounts of work the R.Q. during work tended to become one or even more than one. During the first part of the recovery period there was a further rise in the R.Q. This was followed by a sharp drop to a point well below the resting level. By the time the oxygen consumption was back to the resting level the R.Q. was also usually back to the resting level. The R.Q. of the excess metabolism regardless of the amount of work done was usually in the vicinity of one.

The CO₂ combining power of living mammalian muscle. LAURENCE IRVING, H. C. FOSTER and J. K. W. FERGUSON.

The CO₂ combining power of muscle is shown by simultaneous determination of total CO₂ content and CO₂ tension. The CO₂ content of the dog's gastrocnemius was determined directly by the method of Ferguson and Irving. The CO₂ tension was considered equivalent to that of the unhindered venous outflow from the muscle, and the venous blood was obtained by cannulating the small saphenous vein after ligating all tributaries of the popliteal vein except those from the gastrocnemius. The blood ran directly from the vein along with a bubble of air into a special pipette. In this bubble the CO₂ tension, and consequently the venous CO₂ tension, was determined. When the CO₂ content and tension of one gastrocnemius had been determined, the animal was 1, overventilated or 2, ventilated with 10 per cent CO₂ in order to change the CO₂ tension. The second gastrocnemius was then examined in the same manner as the first.

The figures obtained from each dog gave two points on a curve plotted as total CO₂ content against CO₂ tension. The lines connecting each pair of points for twenty-one dogs coincided closely with an average curve between 30 and 200 mm. CO₂ tension, and showed that the small changes in lactic acid content observed had no significant effect.

The average curve rises less steeply than that for reduced blood and becomes straight at $\text{CO}_2 = 25$ volumes per cent, $\text{Pco}_2 = 55$ mm. It extends from this point about parallel to the curve for venous blood to 45 volumes per cent at $\text{Pco}_2 = 175$ mm. The curve is quite similar to those found by Fenn and Stella for frog muscle.

Studies in absorption from the gall bladder. II. Calcium. C. G. JOHNSTON, J. L. MORRISON and I. S. RAVDIN.

A similar preparation was used as in the previous experiments. Calcium lactate in concentrations from 0.006 to 0.2 per cent was placed in the gall bladder and the concentration and total calcium content studied over a period of hours. Calcium is absorbed from the gall bladder but water is more rapidly absorbed so that the concentration of calcium increases. With the higher concentrations the gall bladder becomes irritated after a time and calcium is then removed from the solution more rapidly. In such instances the concentration of calcium in the solution decreases.

A method for determining the rate of eruption of teeth. FREDERIC T. JUNG and CLAIR L. JOHNS.

In the normal rodent incisor the rate of eruption is in the long run equal to the rate at which the cutting edge is worn off, so that the old method of measuring from the cutting edge to a scratch-mark on the tooth indirectly gives the rate of eruption. But under abnormal conditions the two rates become unequal. Another method that has been used consists in cutting off a tooth and timing the process of regeneration; but it is now known that the eruption of a broken tooth is accelerated and that adaptive changes in the rate of eruption occur in all the other incisors at the same time.

We have worked out a method in which roentgenograms directly give the rate of eruption. A small hole is drilled into the labial, enamel-covered surface of the left lower incisor near the gingival line. Dental x-ray films are exposed at such an angle that the marked convex edge of the tooth and certain landmarks in the mandible itself are clearly visible. Measurements on the film are made with micrometer calipers. Since the tooth is a segment of a circle and since the measurements give chords on the circle, the readings have to be reduced to give the corresponding arcs; this necessitates measuring the radius of curvature of the labial surface of the tooth on the film and then using a numerical table. Although there are further technical difficulties which we have not mentioned, the method is necessary for the study of the effects of very abnormal conditions.

Observations on the overdistended heart. LOUIS N. KATZ and W. A. BRAMS.

A comparison was made of the effect of venesection on the normal and overdistended heart, utilizing methods of high precision and optical recording. It was found, as is well known, that in the normally distended heart venesection decreased the pressure levels and amplitudes of both ventricles, and reduced their volume. In some of the animals, particularly when the blood pressure was low, this effect progressed until the animal died. In others recovery was noted. Hearts were overdistended by infusion of saline or defibrinated blood so that the volume discharge and pressure development of the two ventricles was decreased. Venesection at this time led to a temporary or permanent improvement in the heart's condition

under certain circumstances. When the heart was in poor condition the beneficial effect of venesection was rare.

It was observed that in these overdistended hearts, especially in the right ventricle, a distinct negative wave of some duration was registered. This wave resembles that described by one of us (L. N. K.) in the turtle heart and is taken to indicate the magnification of the suction action of the heart under these special conditions. The reason for the large magnitude of this wave lies in the fact that in these experiments the venous pressure approached the pressure in the pulmonary artery as was shown by direct measurements.

The action of diuretics in the normal individual. NORMAN M. KEITH.

Various diuretic substances were given eleven normal men whose daily food and fluid intake were carefully controlled. The weighed diet was constant from day to day and its content low in water and salt. The fluid intake was kept at a minimum. After three or five days on such a regimen, the urine output reached a constant low volume. A considerable amount of the diuretic substance was then given and the changes in urine, pH, volume, chloride, total fixed base and nitrogen content observed. The diuretics administered were ammonium chloride, ammonium nitrate, ammonium acetate, sodium nitrate, urea, sucrose, organic mercury and theophylline-ethylenediamine. The diuretic effects were most marked after organic mercury, urea and nitrates and minimal after digitalis and ammonium acetate. These results afford a basis for the possible relative usefulness of diuretic agents in the treatment of dropsy.

Observations on the central origin of the sympathetic tone of the eye structures in the cat. ALLEN D. KELLER.

In chronic mid-brain preparations, observed over periods up to three weeks, the sympathetic tone of the eye structures is still present when the animal is aroused. When one cervical sympathetic nerve is cut in such a preparation the presence of the sympathetic tone in the opposite eye structures is readily demonstrated. This indicates that the sympathetic tone of the eye structures is not dependent on a center or centers in the brain-stem cephalad to the mid-brain.

This does not eliminate the possibility that there are cells located here that mediate a tonic or integrative function in an intact animal. However, there is no evidence that necessarily indicates that such might be the case, since stimulation experiments, even if the points are well localized, only indicates synaptic connections, and does not necessarily give any clue in regard to tonic or integrative functions.

The tone that is exhibited in the eye in mid-brain preparations is present only when the animal is aroused by stimulation or by spontaneous activity. This of itself strongly suggests that the sympathetic tone of the *eye structures* is elicited and maintained by afferents that are tonically evoked by receptors in the body during activity or wakefulness.

This does not necessarily indicate that in the intact animal the sympathetic tone is not elicited by humoral avenues or by cells endowed with automatic rhythm of discharge. However, there is no direct evidence that necessarily indicates that such might be the case.

The action of anesthetic drugs on the motor cortex of monkeys. ALLEN D. KELLER and JOHN F. FULTON.

The disclosure¹ that the excitability of the motor cortex is little, if any, impaired under profound surgical anesthesia obtained by means of diallyl barbituric acid ("dial"), has proved of considerable practical importance since it enables one to study the responses of the motor cortex in the course of full and even anesthesia. The question whether other similar drugs give anesthesia without impairing the action of the motor cortex at once presents itself. We have made 25 observations on 16 monkeys and 3 chimpanzees with different drugs and dosages, some under aseptic precautions and others were carried out on animals that were being terminated for other reasons. The following drugs were found to leave the motor cortex active, even when considerably more than the surgical dose was given: "dial," sodium amytal, sodium barbital, chloral hydrate, "avertin."

Morphia, in one instance, caused considerable depression of cortical excitability without giving surgical anesthesia, and "nembutal" (sodium-ethyl, l-methyl, butyl barbiturate) in doses of 25-30 mgm. per kilo (light anesthesia) has little effect on the cortex, but under full surgical anesthesia (35-40 mgm. per kilo) the excitability is depressed, and in one instance was completely abolished. "Nembutal" is a very satisfactory surgical anesthetic for monkeys since they recover within 4 to 5 hours.

Sodium luminal, on the other hand, and luminal itself, cause complete abolition of the responses of the motor cortex in full surgical anesthesia. In a dose of 100 mgm. per kilo, which stupefies the animal without abolishing movement, responses can be obtained; with 150 mgm. it is very greatly diminished and with 200, which is scarcely enough for a surgical operation, responses of the cortex disappear.

The rôle of the cerebral cortex in diurnal sleep in dogs. N. KLEITMAN and N. CAMILLE.

Completely decorticated dogs sleep in the usual manner, but it is rather difficult to arouse them. The main difference between these dogs and normal dogs with respect to sleep is in the diurnal distribution of rest and activity. In the course of 24 hours decorticated dogs have several periods of almost incessant activity alternating with periods of rest. They lead a polyphasic type of existence, resembling that of young puppies or babies, as differentiated from the monophasic type, with one long sleep period, characteristic for older dogs and humans. It would seem that although sleep as such is possible without a cortex, diurnal sleep, as an acquired habit, depends upon the presence of a cortex for its establishment and maintenance.

The effect of cinchophen on the liver of white rats. ROSS M. KNOBLE and H. A. SMITH.

We have attempted to find the effect of cinchophen on the liver of experimental animals. Seventeen female white rats were used. The dosage was 0.025 gram per 100 grams of body weight given subcutaneously. The number of doses given varied between 12 and 32, while the total

¹ Fulton, J. F., E. G. T. Liddell and D. McK. Rioch. Journ. Pharm. Exper. Therap., 1930, xl, 423.

amount of drug a rat received was between 0.641 and 1.748 grams. The cinchophen was given daily in most cases.

All of the rats exhibited liver injuries in some degree. Seven rats died during the treatment.

Macroscopically, a typical liver showed a mottled, yellowish-brown appearance over one-half of the anterior surface of the right lobe, and the posterior surface along the border. The left lobe was extremely mottled on both surfaces, except the area of the gastric impression. There was a marked shrinkage of the mottled area along the border line. On sectioning there appeared to be a subcapsular hemorrhage coinciding with the surface area of discoloration.

Microscopically, the livers showed marked cloudy swelling along the border; a wall of red blood cells along the border and hemorrhage into the surrounding tissue; and necrosis in a few cases.

The 15 control rats showed none of the above changes.

Contributions to the physiology and pharmacology of vomiting. THEODORE KOPPANYI.

During our experiments on medullary centers it became evident that dogs which did not vomit following apomorphine administration because of damage to the vomiting center, did show repeated retching and vomiting following oral administrations of zinc sulphate, copper sulphate and tartar emetic. Indeed, it was possible to abolish vomiting by ablation of an area in the floor of the fourth ventricle, or by local application of morphine sulphate to this aforesaid area, or by systemic administration of five mgm. of morphine sulfate per kgm. of body weight to *subcutaneously or intravenously* injected doses of apomorphine, pilocarpine, tincture of digitalis, but *not to orally* administered irritant emetics.

These experiments carried out in dogs throw further light on the nature of the central control of vomiting in these animals.

Comparison of the effects of estrin,¹ theelin,² gonad-stimulating substance from pregnancy urine,³ and anterior pituitary hormone³ on the basal metabolism, mating, and maternal instincts, lactation and the reproductive system of normal and castrate female dogs. M. M. KUNDE, H. B. VAN DYKE, ZONJA WALLEN-LAWRENCE, OLIVER KAMM and A. J. CARLSON.

These substances all have negative effects on basal metabolism rate and positive effects on the sex behavior and mating instincts.

Sweating response to heat: Variation in total nitrogen, urea, chlorides and sugar in sweat from different parts of the body. OSCAR L. LEVIN and SEYMOUR H. SILVERS.

In a series of 63 experiments performed on 52 patients from the dermatological clinic, it was noted that the perspiration response varied. The temperature of the room and the cabinet was kept constant in all these tests; and the time at which visible perspiration first became evident was recorded.

In cases of pruritis, sweating was delayed; whereas in psoriasis and

¹ Prepared by D'Amour and Gustavson.

² Allen and Doisy crystalline material prepared by Parke, Davis and Company.

³ Prepared by Wallen-Lawrence and van Dyke.

acne vulgaris, with the exception of one case of a negro, sweating was very rapid. We can also corroborate the finding of other observers, (Gans; Pemberton, Cajori and Crouter) that psoriatic lesions do not sweat.

Regional examinations of the total nitrogen, urea, chlorides and sugar in sweat were also performed on the sweat collected from the trunk, feet and legs, and hands and forearms.

The total nitrogen of trunk sweat was found in 53 examinations to vary between 22.5 and 133 mgm. per 100 cc.; that of the feet and legs, in 37 examinations, between 43.4 and 143.3; and that of the hands and forearms, in 19 examinations, between 34.8 and 144.9.

The urea of trunk sweat was found in 50 examinations to vary between 18 and 111 mgm. per 100 cc.; that of the feet and legs, in 38 examinations, between 38.1 and 116.5; and that of the hands and forearms, in 19 examinations, between 21.6 and 120.6.

The chloride content of trunk sweat was found in 42 examinations to vary between 103 and 442.9 mgm. per cc.; that of the feet and legs, in 21 examinations, between 190 and 467; and that of the hands and forearms, in 11 examinations, between 116 and 425.

The sugar of trunk sweat was found in 44 examinations to vary between 6.1 and 25.0 mgm. per 100 cc.; that of the feet and legs, in 34 examinations, between 8.4 and 25.6; and that of the hands and forearms, in 25 examinations, between 11 and 25.0.

A comparative study of the conditioned motor reflex in the rabbit, sheep, goat, and pig. H. S. LIDDELL and O. D. ANDERSON.¹

The aim of our comparative study is to test Pavlov's theory of the conditioned reflex which cannot be accepted at its face value because 1, it is based upon observations of a single species, viz., the dog, and 2, the observations center upon but one effector organ, the salivary gland. It is, therefore, a *special* theory and our purpose is to determine its validity as a general theory of brain action. Satisfactory methods have been devised for establishing conditioned motor reflexes in the above animals using the defensive reaction of the foreleg to an electric shock as the unconditioned response. Leg movement, respiration, and the galvanic skin reaction are graphically recorded. The conditioned reflex is established most rapidly in the pig and more slowly in the goat, sheep, and rabbit respectively. A central feature of Pavlov's theory is the notion of irradiation and concentration of excitation and inhibition in the cerebral cortex. Since salivary secretion continues for several minutes after the presentation of food, evidence for irradiation has been derived from the study of the after-effects of inhibitory conditioned stimuli only. Since the defensive motor reaction in a trained animal is very brief we have been able to demonstrate a phenomenon interpreted as irradiation of excitation in conditioned motor reflexes of sheep. Discrimination of spots about 4 cm. apart on the hind limb can be achieved through training if stimulation of one spot is always followed by a shock to the foreleg while stimulation of the other spot is never followed by a shock. At certain time intervals following the positive conditioned reflex from one skin spot the negative spot regularly gives a positive reaction and the discrimination is disturbed. The time course of this disturbance is being explored in detail and seems to support

¹ Fellow of National Research Council.

Pavlov's hypothesis of irradiation. We are also attempting to demonstrate similar after-effects of inhibitory skin stimuli.

The innervation of the stomach and rectum and the action of adrenalin in elasmobranchs. BRENTON R. LUTZ.

Faradic stimulation of the first sympathetic ganglion and the anterior splanchnic nerves caused extensive contractions of the stomach, beginning in the pyloric region, in *Squalus acanthias* with the brain and cord destroyed. Similar stimulation of the vagus caused moderate contraction confined to the region of the pylorus. Stimulation of the posterior splanchnic nerves gave vigorous contraction of the rectum and adjacent part of the spiral valve.

Adrenalin chloride, 1 in 50,000, and fresh extract of chromophil tissue of the elasmobranch caused a rise in tone and sometimes initiated motility in isolated segments of the stomach of *S. acanthias*, *Raia erinacea* and *R. diaphanes*. The same substances, however, caused a marked fall of tone in isolated segments of the rectum and posterior part of the spiral valve. Extracts of liver and spleen had no effect on pieces of the stomach and rectum.

Influence of barometric changes on potency of digitalis for cats. DAVID I. MACHT.

For the past five years the author has been investigating the influence of various factors on the reliability of the cat method for digitalis assay, and more particularly the influence of changes in the barometric pressure of the atmosphere and of other meteorological phenomena such as humidity, etc. A tabulation of the data obtained from numerous experiments leads to some extremely interesting conclusions. It was found that whenever a given specimen of digitalis tincture was assayed on cats at sea level, as, for instance, in the City of Baltimore, the average lethal dose for the cats differed from the average lethal dose obtained with the same tincture when the assaying was carried out on cats at a higher altitude as, for instance, in the mountains. The minimal lethal dose obtained at a higher altitude, that is, under lower barometric pressure, was always smaller than that obtained at sea level; in other words, the digitalis was more toxic at the more elevated geographical location.

Three sets of experiments on three different expeditions were performed by the author and his assistants in the Blue Ridge Mountains of Western Maryland. One set of experiments was carried out for the author by a colleague in the Rocky Mountains and two sets of experiments were performed by another colleague in the Tyrolian Alps. In each set of experiments, when the digitalis tincture was assayed by intravenous injection on a number of cats according to the Hatcher method, the average minimal lethal dose obtained was smaller than that obtained with another portion of the same tincture in Baltimore, where similar experiments were performed at nearly the same time. In other words, the digitalis tincture seemed to be more potent or more toxic at the higher altitude, where the barometric pressure was considerably lower than in Baltimore.

Systematic observations have been kept with a barograph and a hygrometer in the author's laboratory on atmospheric conditions, and whenever a digitalis assay on cats was performed, the barometric pressure, humidity, and weather conditions were carefully noted. It was observed almost

invariably that when given tinctures of digitalis were assayed on cats in fair weather and at a high and steady atmospheric pressure, the figures obtained were usually greater than when the same tinctures (which had been kept in the ice chest in the dark) were assayed on days when the barometer was low and the weather was cloudy. It was noticed, furthermore, that very marked fluctuations in the toxicity of digitalis occurred during a violent storm, especially when the barometer was rapidly falling.

The differences in the minimal lethal dosage obtained at higher and lower altitudes, as well as during severe meteorological disturbances, are not due to changes in the digitalis itself but must be ascribed to physiological changes occurring in the cats, more particularly in circulation and respiration, because the same tinctures, or other digitalis preparations, were invariably used in any two given sets of experiments.

Marked changes in humidity, artificially produced in the laboratory, seemed also to influence the lethal dosage of digitalis for etherized cats. The most remarkable changes, however, were noted in connection with studies on barometric pressure. Further observations are in progress. The results already obtained, however, are of interest not only from a purely scientific point of view but also for practical purposes because, in order to determine the potency of any given specimen of digitalis, it is obviously necessary to take into account the geographical location of the laboratory in respect to its altitude and the state of the weather.

Relation of method of slaughter to the toxicity of blood sera and muscle extracts.

DAVID I. MACHT and HELEN M. COOK.

Solutions of blood sera and muscle extracts in physiological saline were studied by the authors on the growth of living seedlings of *Lupinus albus* according to phytopharmacological methods described by Macht and his co-workers in numerous previous papers. Experiments were made with the blood and muscle, respectively, obtained from a large variety of animals and in all the experiments no drug was employed except ether for general anesthesia, which anesthetic was completely volatilized after the extracts had been prepared. Blood and tissues were obtained from the following classes of animals: fish, frogs, mice, rats, guinea pigs, rabbits, cats, dogs, hens, pigeons, oxen, calves and sheep. Comparative studies on blood were made after arteriotomy and complete exsanguination, on the one hand, and decerebration and various injuries to the brain, on the other hand, and also after fatal ether asphyxia and after electrocution. It was found that blood obtained from asphyxiated animals, such as rabbits, dogs and cats, was more phytotoxic than blood obtained by arteriotomy without extreme asphyxia. It was further found that blood obtained after decerebration or severe injuries to the brain was much more toxic for living plant protoplasm than that obtained after either arteriotomy or after asphyxia. This was found to hold good for every species of animal studied.

Extracts of muscle tissue were made by carefully rubbing up the muscle substance with a plant physiological saline solution of definite composition and carefully filtering, always under carefully controlled conditions. It was found that for any given species of animal, a definite phytotoxic index or inhibition in the growth of *Lupinus albus* seedlings was obtained. When the animal was killed by bleeding from severed arteries, the muscle extracts were less toxic than muscle extracts obtained from animals killed in other ways. Thus, muscle extracts after asphyxia were more toxic than

after arteriotomy. The most striking differences, however, were noted on comparing muscle extracts of different animal species obtained after arteriotomy and decerebration. In such cases the differences in phytotoxic activity were often as high as 50 per cent, the muscle extracts obtained after injuries to the brain being invariably more toxic than those obtained after arteriotomy. Further experiments revealed that this variation in the toxicity could not be explained altogether by the different amounts of blood remaining in the two muscle specimens after exsanguination and decerebration, respectively. There were indications that injury to the brain sets free some toxic bodies, the chemical nature of which has not yet been determined, which are carried by the blood stream to the muscles and render extracts of such muscle tissue more toxic, even in a comparatively short time after death. Muscle extracts from electrocuted animals (under ether) were found to be also more toxic than extracts of muscle obtained from other individuals of the same species, and even from muscle tissue obtained (under ether) from the same individual before electrocution.

Phytopharmacological examination of stomach washings from various clinical conditions. DAVID I. MACHT and MOSES PAULSON.

Employing Macht's phytopharmacological methods, a systematic study of over one hundred and twenty-five patients in respect to the toxicity of their stomach washings for the growth of living seedlings of *Lupinus albus* was made. A uniform procedure or technique was followed in all the experiments. A patient's stomach was washed early in the morning before any food was taken with 100 cc. of distilled water. The hydrogen ion concentration of the pumped-out fluid was then determined, and the specimen was afterwards studied by phytopharmacological methods. A two per cent solution of the "washing" in each case was made in a standard plant-physiological saline solution, and the growth of the straight, well-defined roots of *Lupinus albus* seedlings at a temperature of 20°C. for twenty-four hours in the dark was carefully noted. These were compared with control seedlings, grown under exactly the same conditions. A large variety of clinical cases was examined in this way with the following results.

The average phytotoxic index or coefficient of growth obtained from a hundred patients was 87 per cent, as compared with the growth of normal controls in plant-physiological solution without the admixture of any stomach washings. The readings varied from about 80 to 100 per cent. It was found that stomach washings from cases of carcinoma showed no difference in toxicity from specimens obtained in other gastric conditions. Studies on the variations in the hydrogen ion concentration revealed that the toxicity of the solutions bore no relation whatever to slight differences in their hydrogen ion concentration.

The most interesting findings, however, were connected with cases of pernicious anemia and of achylia. Phytopharmacological examination of stomach washings from eight cases of pernicious anemia, with the invariable concomitant achylia, gave an average index of 61 per cent. On the other hand, phytopharmacological examination of seven cases of true or functional achylia with no evidence of pernicious anemia or any other organic disease gave an average index of 89 per cent. The seven cases of achylia were of a severe type which failed to respond with acid secretion to injections of histamine. The difference in the readings ob-

tained between pernicious anemia, on the one hand, and the essential achylia, on the other, promises to be of diagnostic value. The present study in regard to pernicious anemia is of interest also in connection with findings made previously by Macht and reported in these Transactions last year concerning studies on the toxic reactions of spinal fluid from pernicious anemia patients. The concomitant toxicity of blood serum, spinal fluid and stomach washings for plants may serve to throw some light on the etiology of this baffling disease.

Restoration of pancreatic secretion by peptone and histamine. MARGARET E. MACKAY and S. G. BAXTER.

The repeated injection of HCl into the duodenum of dogs and cats under certain conditions soon fails to activate the pancreatic secretion. Rasenkov and Koschtajanz found that the secretory action of HCl could be restored at this point by the introduction into the intestine of peptone or acid digests of protein. The amino acids did not have this effect.

We found that the secretory action of HCl could be similarly restored by using a solution of histamine. Since histamine is usually present in commercial peptones and presumably in the protein digests, the restorative effect in these cases might be partly due to histamine. During the course of our experiments it was noted that histamine is absorbed from the small intestine in acid solution (0.2 per cent HCl) but not in aqueous solution.

Observations on carbohydrate metabolism in the dehepatized animal. FRANK C. MANN and S. GLEN MAJOR.

The rapid decrease in the concentration of sugar in the blood and the glycogen in muscles following removal of the liver indicates fairly rapid and uniform utilization of carbohydrates in the dehepatized animal. That this utilization can be increased is demonstrated by the fact that increasing amounts of glucose are necessary to maintain normal blood sugar in the dehepatized animal. The process of utilization of carbohydrates in the dehepatized animal is not only of interest from the standpoint of the physiology of the liver, but is probably of value in unravelling the mystery of insulin and the fate of carbohydrates in the diabetic patient. It has been shown that the respiratory quotient of the dehepatized animal is increased and that the specific dynamic action of glucose is enhanced. However, these observations are not sufficient to indicate definitely the process of utilization of carbohydrates in the dehepatized animal. In order to study the effect of depletion of the glycogen stores as a step in the study of its utilization in the dehepatized animal various methods were tried for the reduction of glycogen in the normal animal. These included a study of the effect of ether, *iso*-amylethyl barbituric acid (amytal), and epinephrine on the glycogen in the liver and muscles. It was found that ether anesthesia decreased the glycogen in the liver and muscles. *Iso*-amylethyl barbituric acid caused but a slight change in the glycogen in the liver and muscles. The administration of effective doses of epinephrine always decreased glycogen in the muscles, but the effect on that in the liver was variable; usually it was decreased also.

The same measures that produced decrease of glycogen in muscles in the normal dog also appear to produce a definite decrease of glycogen of the muscles after complete removal of the liver. In the depancreatized animal the glycogen lost in the muscles (especially after the administration

of epinephrine) appears in the urine as glucose. In the dehepatized animal additional glucose does not appear in the blood or urine after epinephrine and the changes in lactic acid of the blood and urine are not of sufficient magnitude to account for the loss of glycogen in the muscles. After hepatectomy there is a variable increase in the lactic acid of the blood and urine, but large amounts of lactic acid disappear following injection of this substance. Glycogen may be formed in the muscles of the dehepatized dog following administration of glucose and epinephrine in amounts which allow the glycogen of the muscles of normal dogs to decrease to very low levels.

Thermoelectric temperature studies in visceral organs. FRANK MARESH.

Copper-constantan thermocouples of no. 37 cotton insulated wires were further insulated with cellophane and inserted into the visceral organs of nine rabbits. Temperatures were observed simultaneously, or nearly so, for as many as 13 hours in the kidneys, liver, spleen, suprarenals, stomach, rectum and muscles of nine rabbits anesthetized with ether during the operative procedures and amytal for the post-operative period. With the exception of muscle temperatures, the temperatures in the visceral organs agree to within $0.5^{\circ}\text{C}.$; muscle temperatures were lower than the rectal and organ temperatures in most cases. Marked temperature decreases were observed with ether, and less with amytal. The rate of heat loss from an anesthetized rabbit is about the same as for an expired or dying one.

The redistribution of water in some toxic and neurogenic fevers. H. T. MARSHALL and B. F. AYDELOTTE.

Fever since the time of Leyden has been thought by many to entail both retention and redistribution of water. Hitherto no specific organ has been shown to be the seat of storage.

The onset of experimental fever is accompanied by concentration of the blood (Barbour). The amount of this blood concentration which we find constantly in cocaine fever rabbits of about two kilos weight, suggests a water loss from the circulation of about 6 cc. This water might simply leave the body in extra evaporation, but in four out of five animals significant increase in insensible loss was not found until the peak of blood concentration had been reached. Likewise the body cavity fluids were not found increased.

On killing rabbits of like age and species at various stages of cocaine fever, we found no significant change in the water content of skin, muscle, kidney or brain. But the water lost from the blood can all be accounted for by storage in the liver. The percentage solids of this organ in nine normal rabbits averaged 28.14 ± 0.58 (standard deviation) while the average for twelve cocaine fever rabbits was 26.03 ± 0.29 . From this it can be calculated that the two kilo animal stores about 8 cc. of extra water in the liver.

The results found for cocaine fever have been confirmed by work on other fevers, namely, those due to beta-tetra-hydronaphthylamine and horse-serum anaphylaxis.

Fevers of central origin such as "heat puncture fever" and "cold fever" in rabbits have not hitherto been studied with reference to the water exchange. We now find such fevers also accompanied by marked blood concentration and increase in liver water. (Likewise heating the basal ganglia reduces the liver water and dilutes the blood.)

By severing the hepatic artery and its accompanying nerves we find that the liver becomes still more edematous than in any of the fevers mentioned. This is accompanied by an abnormally high blood specific gravity which cannot be influenced by our brain puncture even when reinforced by cold.

The activity metabolism of a single muscle in situ. E. G. MARTIN, J. FIELD, 2d, V. E. HALL and S. M. FIELD.

In dogs anesthetized with amytal the content of arterial blood in oxygen and lactate was determined. The entire venous outflow from one gracilis vein for about five minutes was collected and the quantity noted. This blood was also analyzed for oxygen and lactate content. The muscle was then stimulated through its obturator nerve for about one minute by rhythmically repeated tetanic shocks; the entire venous outflow during the period of exercise was collected and the quantity noted. This blood was also analyzed for oxygen and lactate content. The tension developed by the muscle at each contraction was determined isometrically. Immediately after the end of the exercise the worked muscle was frozen *in situ* with carbon dioxide snow, excised and ground to powder in liquid air. The lactate content of the muscle and its weight were determined. Just prior to exercising the gracilis an adjoining resting muscle was frozen, excised, and analyzed similarly to give an approximate lactate base line. The main data were thus assembled for a description of the activity metabolism of an individual muscle during a brief period of work. Results to date are as follows:

The average oxygen consumption per 100 grams of muscle per minute *before exercise* was 0.75 cc.; the average lactic acid production was negligible. The average oxygen consumption per 100 grams of muscle per minute *during exercise* was 3.4 cc.; the average lactic acid production (including both the amount given off into the venous blood and the increase in quantity present in the muscle) was 82.5 mgm.

Oxygen determinations were made by the method of Van Slyke¹ and lactates by the method of Friedemann, Cotonio and Shaffer.²

Flexion reflex recorded with muscles in situ. G. P. McCOUCH.

Preliminary results³ were distorted by mechanical errors.

The present method employs a frictionless torsion lever bearing on each arm a flat wound coil of fine enamelled wire, the pair forming an inductorium. The primary coil is in the plate circuit of a high frequency vacuum tube oscillator. The secondary coil connects grid and negative of filament of a rectifying tube with a string galvanometer in its plate circuit. Each lever is designed to meet the mechanical situation of the muscle recorded. In the case of tibialis anticus, in which angle of pull is limited by the annular ligament, the lever is fixed to the tarsus with its shank immediately over the insertion at right angles to the tendon. In the instrument recording gastrocnemius, soleus, and plantaris, the fixed arm forms a flat, hook-shaped guard protecting its shank from contact with adjacent skin and permitting a shift of angle to maintain an approximately rectangular relation of tendon to lever shank.

¹ Van Slyke, D. D. and W. C. Stadie. 1921. Journ. Biol. Chem., xlix, 1.

² Friedemann, T. E., M. Cotonio and P. A. Shaffer. 1927. Journ. Biol. Chem., lxxiii, 335.

³ This Journal, 1925, lxxii, 217.

Preparation of tendons includes ligation at insertion and at a point far enough up to make the distance between ligatures measured after the lever is in place approximate that before section of the tendon. Ligatures are soaked in 20 per cent cocaine hydrochloride before use. The skin incision is sutured leaving a button hole for the tendon.

Movement of ankle is recorded by a rubber bulb fixed to its flexor surface with air transmission to a Frank capsule.

Results: In weak and moderate flexion reflexes elicited from foot pad or hamstring nerve, tension of tibialis anticus rises *pari passu* with flexion of ankle. Tension of the extensor group remains constant. Weak flexion reflexes from nerve stimulation show recruitment; those from foot receptors are *d'emblée*. Decerebrate cats were employed throughout.

Lactic acid and the coronary circulation. DANIEL A. MCGINTY.

In an effort to discover additional information as to the distribution and disposal of lactic acid in the body, a study was made of its content in simultaneous samples of arterial and of coronary venous blood of the beating heart during alterations in coronary volume flow of blood.

In 137 of 140 pairs of samples in 13 dogs under a variety of experimental conditions, coronary venous blood contained less lactic acid than arterial blood by amounts ranging from within the analytical error up to 23 mgm. per cent. Tabulation of results indicates no parallelism between the extent of absorption of lactic acid as indicated by the arterial-venous differences in the coronary system and the arterial lactic acid levels.

Stimulation of the stellate ganglion caused a decrease in the arterial venous differences during the period of increased coronary flow. Vagal stimulation had the reverse effect during the fall in volume flow.

Following rapid intravenous injection of moderate amounts of pitressin and during the moderate decrease in coronary volume flow, increased lactic acid absorption from the blood was observed. With larger injections following which a considerable reduction in volume flow occurred a reduction in absorption took place, and in 3 experiments an initial pre-injection absorption was replaced by an outward diffusion of lactic acid into the blood.

With moderate changes in coronary volume flow during vagal and stellate stimulation and with small doses of pitressin, the percentile absorption of lactic acid is believed to be primarily dependent on the velocity of blood through the heart capillaries. With more pronounced diminution in flow of blood and a subsequent reduction in oxidations in heart muscle, increased lactic acid production may reduce the gradient between blood and muscle or establish a reverse gradient from muscle to blood.

The effect on the pyloric sphincter of acid in the stomach and in the duodenum.

C. J. MOGAN and J. EARL THOMAS.

For this study dogs were used in which flanged cannulas made of hard rubber had been placed in the stomach and duodenum and allowed to heal into the abdominal wall. The animals were operated upon under ether anesthesia and studied without anesthesia three to six weeks later.

Graphic records of the tonus and contractions of the pyloric sphincter were obtained by the use of the pressure tonometer, previously described by one of us.¹ Using this instrument, one records the pressure required

¹ Thomas, J. Earl. 1929. This Journal, lxxxviii, 498.

to force a constant stream of air through the pylorus. The air is conveyed through rubber tubes placed in the stomach and duodenum, connected by a flexible section lying in the pyloric orifice.

Generally, the tubes were placed, via the cannulas, on the day before observations were made. There was no evidence of digestive or nutritional disturbance resulting from the operations or the presence of the tubes. The animals ate heartily when food was offered during an experiment and gastric evacuation proceeded in a normal manner so far as we could determine.

Tenth normal hydrochloric acid was used as a stimulus. It was delivered as near as possible to the pyloric sphincter on either side through small accessory tubes tied to the air tubes. Experiments were performed with the stomach empty, partly filled with water, and after feeding dog biscuit and milk. The food was especially suited to this study in as much as it greatly delayed, or entirely prevented, the spontaneous appearance of free HCl in the stomach.

Injecting acid into the duodenum commonly caused a brief increase in the tonus of the sphincter accompanied by a series of rapid contractions, followed by a more prolonged cessation, or decrease in extent, of contractions and frequently a moderate decrease in tonus. We were unable by any means at our disposal to prolong the increase in tonus but increasing the amount of acid did prolong proportionately the period of inhibition. In our experience the predominant effect of HCl in the duodenum was to decrease the extent and constancy of the contractions. The sensitivity to acid was markedly decreased by taking food and by the previous administration of acid. Threshold amounts ranged from 2 to 20 ml. according to conditions. Control injections of tap water gave results that were negligible in comparison with the effects of similar amounts of acid.

Injecting acid into the stomach in amounts ranging from 4 to 40 ml. gave entirely negative results, or a moderate increase in the tonus of the sphincter, except when the stomach was partly filled with water. Under this condition free HCl appeared in the duodenum shortly after its injection into the stomach and the results occasionally resembled those described for the duodenum, including the decrease in contractions and tonus.

The inhibitory effect on the sphincter following the injection of acid into the duodenum we regard as probably incidental to a general inhibition of the stomach. Experiments now in progress to test this hypothesis have given only results in accord with this interpretation.

So far as we know, an inhibitory effect on the sphincter due to acid in the duodenum, has not previously been described. The other results are similar to those obtained by Carlson and Litt¹ in anesthetized animals.

The continuous secretion of pancreatic juice in the dog. M. LAURENCE MONTGOMERY and LESTER R. DRAGSTEDT.

A fistula which permitted the collection of the entire external secretion of the pancreas was prepared in a number of dogs. The method has been previously described by the authors. Hourly collections of pancreatic juice were made for periods of 60 to 90 hours after the last meal. During this entire period there occurred a secretion of pancreatic juice ranging in amount from 5 to 70 cc. per hour.

¹ Carlson, A. J. and S. Litt. 1924. Arch. Int. Med., xxxiii, 281.

Some changes in blood gas content and capacity during hyperthermia induced by the high frequency electric current. E. S. NASSET.

Experiments were made with anesthetized dogs using a current of 250 to 4000 milliamperes and a frequency of 1,000,000 cycles. The rectal temperatures in some cases reached 45°C. before death ensued. The respiration was increased 10 to 25 times the normal rate. The CO₂ content of whole venous blood and of plasma was in several cases reduced 40 per cent. The CO₂ capacity of whole blood was diminished. The O₂ content and capacity of venous blood showed a very marked increase.

The determination of total transformation of energy by means of the insensible loss of weight. L. H. NEWBURGH.

Two groups of persons were each fed a diet of known composition. The first group was made up of diabetics who had been receiving the same diet for many months, and whose weight had shown no change. The second group, composed of normal individuals, each received diets intended to just meet the energy output. It is safe to assume for both groups that the transformation of energy was the same as the potential energy of the food.

The insensible loss of weight of subjects thus prepared was determined for intervals of 24 hours' duration, or longer. From this value the weight of insensible water was calculated by correcting for the gaseous exchange. The heat removed from the body by the evaporation of this water was found to be very close to 24 per cent of the available energy of the diet. This is in striking agreement with determinations made in the calorimeter for short periods. Thus the insensible loss of water from the body affords an accurate and simple method of determining the total transformation of energy in the organism.

The specific gravity of the blood of emotionally excited rats. L. B. NICE and A. H. SIMONS.

The average specific gravity of the blood of 31 normal rats in the quiet state was found to be 1.058, while after excitement (anger and pain) the same rats' blood averaged 1.067. This gives a difference of 0.009 for these rodents.

Is the rotation of the crystalline style in the clam a substitute for gastric peristalsis? T. L. PATTERSON and ELEANOR S. BOONE.

The large gaper clam (*Schizothaerus nuttallii*) was used and the investigation was conducted at the Hopkins Marine Station of Stanford University. The shell of this exceptionally large, bivalve mollusk attains a length, antero-posteriorly of eight or nine inches and the adult form when the neck is fully extended measures around thirty inches. After etherization of the animal, the balloon was passed through the inhalent siphon, thence through the mantle cavity and mouth into the stomach. The animal was then placed in a vivarium provided with running sea water. A silk thread attached to the tip of the foot and running over pulleys to a recording lever completed the arrangement.

Studies made on the peristaltic activity of the stomach of this animal by the balloon method before and after surgical removal of the crystalline style demonstrates unequivocally that the rotation of the style is not a substitute for gastric peristalsis. This is contrary to Nelson, who maintains that the style among other things functions in this way. The gastric

contractions after removal of the style are practically identical with those obtained when it is intact and in many animals they even become more vigorous in its absence. These contractions are continuous both in the presence and in the absence of the style and they show a definite relationship to the gastric activity observed in various gastropods, which possess no crystalline style, namely, *Haliotis rufescens* (Abalone); *Ariolimax californicus* (Giant slug); *Tethys californica* (Sea hare). The contractions are not due to muscular movements of the foot since they go on uninterruptedly when the foot is quiescent as is shown graphically by simultaneous records recorded from the stomach and the foot. Therefore, the musculature of the stomach of this animal produces the gastric contractions which places it in the same physiological category as the stomachs of other vertebrate and invertebrate animals studied.

Variations in the susceptibility of cats to absinth and hypotonic solutions.

F. H. PIKE.

About one hundred control observations on the minimal convulsive dose of absinth in cats not subjected to any previous experimental procedure, have been plotted, first as a statistical basis of comparison for animals which have been subjected to some experimental procedure before the injection of absinth, and second, as a basis of control for further procedures on the same animals. The distribution curve shows two modes—one at about 0.02 cc. of the standard solution per pound of weight. The curve then falls to its low point at about 0.023 cc. per pound. It then rises gradually to reach its second and highest mode at about 0.03 cc. and then falls to base line at about 0.04 cc. A third small group, since the condition occurs only rarely, has a minimal convulsive dose of about 0.045 cc. The middle group may be taken to represent the conditions in healthy normal cats. In the lower group, a persistent thymus, with thin walled veins which bleed freely, or an enlarged spleen, or both, are almost constant findings at post mortem. Healed ruptures of the spleen and healed fractures of the skull are occasionally found. The statistical reports have been based upon animals without obvious evidences of cranial trauma. No obvious pathology or physical peculiarity has been found in the few animals at the third and highest mode of 0.045 cc. I have no explanation for the difference in the susceptibility of the three types to a convulsant agent.

A further peculiarity appears to be relatively constant, namely, that the animals of the first group withstand intravenous infusion of hypotonic fluids better than those of the middle group, the amounts of fluid ranging from 20 to 30 cc. per pound in the lower group and 10 to 15 cc. per pound in the middle group before the animal succumbs to the combined effect of the fluid and a low dose of absinth.

Satellitosis and neuronophagia in the cat's brain from large doses of bromide.

F. H. PIKE and JOHN NOTKIN.

In connection with the recent interest in the effects of bromides, and Foerster's observations on the effects of large doses of bromides in the human subject after brain operations for the relief of convulsions, we may mention that satellitosis, and even neuronophagia, of the cortical cells in the motor and other regions of the cat's brain may occur from bromide feeding alone. The administration of 1.1 gram of sodium bromide a

day for a period of two weeks or more is usually sufficient to bring on this condition. Such animals succumb more readily to absinth than control animals. This decreased resistance to convulsant agents persists for at least a month after the feeding of the bromide is stopped. How much longer the effect persists, we do not at present know. Some cellular damage seems to occur when 0.5 gram of sodium bromide is fed daily, as is shown by the decreased resistance to absinth. The most favorable results from the point of view of resistance to absinth followed doses of less than 0.5 gram a day.

Intravenous injection of potassium boro-tartrate has been effective in raising the minimal convulsive dose of absinth as much as fifty per cent above the control dose, i.e., the minimal convulsive dose in the same animal before the injection of borax. The greater the amount of the borax injected, the more nearly does the minimal convulsive dose approach the lethal, which is in general less than in control animals.

A comparative study of skeletal structures with reference to the effect of age upon the inorganic composition. SAMUEL E. POND.

The object of the experiments (reported in part) consists in a comparison of data which have been secured by a comprehensive analysis of skeletal solids from acutely killed animals. Different methods of preparation of the skeletal structures have been studied to permit closer correlations of data on biological and functional grounds. Analytical methods for quantitative study have been adapted from recognized chemical procedures, except the method for magnesium which depends upon the stable state of its oxide (prepared in routine from oxalate solutions). The methods have been compared with respect to a considerable range of biological material, including embryonic and adult skeletons, animal and human teeth, crustacean carapaces, and cycloid scales.

Although incomplete as to critical ages in some cases there are two results which stand out when data are arranged with respect to increasing age, viz:

1. Ca/Mg ratios are in reverse relation to PO_4/CO_3
 - a. Ca/Mg in bone increase as PO_4/CO_3 diminish
 - b. Ca/Mg in teeth, cycloid scales, and integumental structures, in contrast to bone, diminish; whereas PO_4/CO_3 values increase.
2. Inorganic residual $\text{P}_2\text{O}_5/\text{CaO}$ are higher in value for all exo-skeletons than for endo-skeletons, indicating, in the former, more acidic components than in the latter.

A comprehensive procedure for comparative, biological study of the skeletal-solids in animals, with particular reference to the quantitative determination of changes in the inorganic constituents effected by increase in age. SAMUEL E. POND.

Methods and criteria have been tested and applied in an elementary, systematic study of biological tissues to determine the limits of variations in the composition of the inorganic skeletal solids comprising endo- and exo-skeletons in different animal phyla. Standard procedures have been but slightly modified, except in regard to: 1, Preparation of samples for biological, chemical and physical study on the same substances with minimum of structural and compositional alteration; 2, devices for determination of total CO_2 of embryos and of gummy materials which other-

wise foul the Van Slyke manometric gas-analyzers in routine work; 3, arrangement of analyses to permit (on one and the same sample) gravimetric, gasometric or volumetric analysis of CO_2 , P, Ca, Mg, Na and K; 4, precision methods of magnesium determination adaptable to gravimetric, volumetric and gasometric analysis (interchangeably) by means of the stable MgO from oxalate, chloride and acetate solutions.

Modifications of the method employed by R. G. Hurlin have been found suitable for the preparation of a large range of marine skeletons, embryonic skeletons, and bones of land animals. This consists in the removal of flesh from the acutely killed, skinned and eviscerated animals. Alteration of the skeletal structures have been studied in sterile, "inactive" trypsin-defleshing operations and also by mixed or pure bacterial, lytic operations. The skeletons are freed from agar and flesh over steam, subsequently immersed, only in hot water and dried in vacuo. Whole skeletons or individual bones, cycloid scales, etc. have been ground, screened and sampled with the aid of a miniature Wiley Mill. Selected parts of skeletal structures such as healed-fractures, sutured bones, and sections of undecalcified materials are cut by rotary, slitting-saws of special design from bones and teeth *in situ*. For such purposes the tissues are embedded in an adhesive, quick setting, easily melted mixture of selenium and sulfur. Portions of such preparations have been employed in an attempt to study chemical, physical and histological differences in different areas of calcified and ossified structures. The data from analyses are given elsewhere and methods have been prepared for publication.

Erythrocytes and oxygen as factors in determining the permeability of animal tissues in general. MARSCHELLE H. POWER, HIRAM E. ESSEX and JOHN M. ORT.

We have shown previously that when the erythrocytes in the circulation of dogs and rabbits drop much below 2.0 cc. per 15 cc. of the circulating fluid, considerable gum acacia will enter the heart either when it is in the intact animal or when it is excised and perfused. Similar studies show a like general tendency throughout the body, as indicated by analyses of urine and of tissue from the lung, spleen, kidney and liver. Further evidence indicates that the oxygen-carrying capacity of the erythrocytes is the factor involved. Brain tissue may be an exception, since even in animals with only a few erythrocytes in the circulation, and with extreme anoxemia, very little gum acacia could be recovered. For tissues other than from muscles, the plasma content of the circulating fluid is also a factor in determining permeability.

Observations on responses limited to the motor unit. F. H. PRATT.

Direct and indirect fibre response and the widespread axone reflex, in the terminal nerve-musculature of the frog's retrolingual membrane, have been brought to immediate observation through a recently described method¹ of microscopical transillumination, wherein single nerve fibres with the innervated muscle fibres can be selectively stimulated without recourse to dissection or injury. Completely conducted maximal response is apparently obligatory upon all parts of a motor-unit, irrespective of the collateral excited or the direction of conduction. Persistent stimulation of the nerve

¹ Pratt and Reid: Science, 1930, lxxii, 431.

shows continuous as well as discontinuous fatigue of the muscle fibre, but in the former case there is no restitution of diminished contractility on direct excitation. Gradation by stimulus-frequency change is possible up to complete tetanic fusion. The directly and indirectly stimulated muscle fibre is subject to the typical veratrine contracture, which strongly resembles that induced by mechanical injury. Curarization effects its disjunction without loss of contractility. Arborization of collaterals into axones of successively diminishing calibre is correlated with successive rise of threshold. The detail of the motor end-organs can now be demonstrated during an experiment through the addition of vital methylene blue to the immersion fluid.

Changes in muscle glycogen accompanying physical training. H. A. PROCTER and C. H. BEST.

Healthy active dogs have been trained to run upon three legs. After certain periods of training the animals were gently anesthetized with amyntal and corresponding muscles from the trained and relatively untrained hind legs were removed for the determination of their glycogen content. In a series of eight dogs, trained for periods of from seven to sixteen days, the trained muscles had glycogen contents of from $1\frac{1}{2}$ to 2 times as high as that of the bilaterally symmetrical untrained muscles. In a second group of 8 animals, trained from twenty to forty-two days, the glycogen content of the trained muscles was found to be somewhat higher than the untrained in two experiments. In the other cases there was either no change or a slight decrease in the glycogen content of the trained muscle. These results suggest that there is an optimum period of training for the production of increased glycogen content of muscle and that the accumulation of glycogen is not an essential concomitant of increased ability to perform exercise.

Effect of carbohydrate administration on gastro-intestinal motility. J. P. QUIGLEY and W. R. HALLARAN.

In considering the chemical control of spontaneous motility of the empty stomach it has been indicated that motility varies inversely as the blood sugar level.

We have reinvestigated the modifications in motility of the gut which follow the administration of carbohydrates. Intravenous injection of glucose in doses from 1 to 25 grams did not modify spontaneous gastro-intestinal motility. This is contrary to the report (Bulatao and Carlson) that such injections inhibit gastric hunger contractions and also to the observation that intravenous glucose will produce complete inhibition of the stomach, ileum and colon when this motility has been augmented by insulin administration. Intravenous administration of lactose (or saline) likewise does not modify spontaneous gastro-intestinal motility.

Introduction of glucose or lactose into the stomach under conditions permitting rapid absorption produced inhibition of spontaneous gastric hunger contractions (saline administration is without effect). We have not inhibited gastric motility by the introduction of glucose into the colon or terminal ileum.

These results show a striking difference between spontaneous gastro-intestinal motility and that induced by insulin administration and also show that under certain conditions motility of the gut is not inversely proportional to the blood sugar level.

The effect of alpha-lobelin on the blood sugar level of adrenalectomized albino rats. F. S. RANGLES.

Continuing the observations on alpha-lobelin hyperglycemia in various animals, published in 1928 by King, Hosmer and Dresbach, experiments were done on albino rats. The rats were divided into three groups: normal, adrenalectomized, and control operated rats. Blood sugar determinations were made from tail blood. Then 22 to 44 mgm. of alpha-lobelin per kgm. were injected intraperitoneally and after 15 to 20 minutes a second blood sugar determination was made. In some instances the liver glycogen was determined also. Of the normal rats one group of 23 showed an average increase in blood sugar of 68.5 per cent, a second group of 13 showed an average increase of 65.7 per cent, with a liver glycogen of 1.35 per cent. Of the adrenalectomized rats, one group of 28 animals, 3 to 7 days after operation, showed an average increase of 10.5 per cent in blood sugar. A second series of 20 animals, 27 to 40 days post-operatively, showed an average increase of 18.6 per cent, with an average liver glycogen of 1.62 per cent. A third group of 16 animals, 36 to 56 days after operation, showed increase of blood sugar of only 3 per cent, with a liver glycogen of 2.41 per cent. The control operated animals were those in which the adrenals were exposed but not removed. Of these one group of 11 animals, 14 to 43 days post-operatively, showed an average increase of 47.1 per cent and a liver glycogen of 0.45 per cent. A second group of 8 animals, 27 to 29 days after operation, showed an average increase of 46.5 per cent and a liver glycogen of 4.02 per cent. From our results it appears that: 1, operative procedures in rats may markedly reduce the liver glycogen over a period of 2 to 3 weeks; 2, in certain experiments on the liver glycogen and blood sugar content it is not safe to use rats under 3 weeks following adrenalectomy; 3, alpha-lobelin induces a markedly less degree of hyperglycemia in adrenalectomized rats, in good condition and with available liver glycogen, than in normal ones; 4, the experiments indicate that epinephrine is a factor, but not the only one, in alpha-lobelin hyperglycemia; another may be cortical.

A comparison of the effects of tyrosine, diiodotyrosine, diiodothyronine and thyroxine upon metabolism. DAVID RAPPORT and ATTILIO CANZANELLI.

These substances were injected intravenously into dogs and the gas exchange of the animals studied. Tyrosine, in doses of 18-35 mgm. per kilo, caused no rise in heat production, but with larger doses (45-70 mgm. per kilo) a definite increase was obtained. The increase due to diiodotyrosine was 6-8 times that of tyrosine; that of diiodothyronine about 25 times that of diiodotyrosine; and that of thyroxine 20 times that of diiodothyronine. The effect of all of these substances was manifested on the day of the injection, that of tyrosine reaching a maximum in the first observations (about one hour after injection). In the case of diiodotyrosine and diiodothyronine the increased metabolism also began almost at once, but the rise to maximum was delayed. In the case of thyroxine, the first large rise in metabolism did not occur until 4 to 5 hours after injection of 0.5 mgm. per kilo, and the maximum was not reached for 50 hours.

Studies in absorption from the gall bladder. I. Sodium chloride. I. S. RAYDIN, C. G. JOHNSTON, J. H. AUSTIN and C. RIEGEL.

The gall bladder of the dog was used in these experiments. It was

isolated from any ductal connection, but its blood vessels and lymphatics remained intact. A catheter was inserted into the common duct and thence through the cystic duct into the gall bladder. The experiments were done on anesthetized animals. The following studies have been carried out: 1, the absorption of water; 2, the absorption of chloride; 3, the fate of base; 4, the CO_2 content of the solutions studied during different periods of absorption. Hypotonic, isotonic, and hypertonic solutions of NaCl have been used as well as mixtures of sodium chloride and sodium bicarbonate.

Hypertonic solutions decrease in their chloride concentration approximately to the blood level for chlorides. Hypotonic solutions increase in their concentration to the same level. The CO_2 content increases as the chloride concentration decreases and at times reaches a concentration two to three times that found in the blood plasma.

Intravenous use of viosterol in parathyroid tetany. C. I. REED and LINDON SEED.

The value of viosterol as an antitetany agent in dogs has been demonstrated by Brougher, by Jones and others. Reports of clinical results have not been so uniformly favorable. We have demonstrated that this material is effective intravenously. Intravenous administration in four parathyroidectomized dogs and five clinical patients and in a large number of normal subjects, both dogs and human subjects, has demonstrated the following points: 1, small doses of a highly potent preparation will protect against tetany; 2, the effect is more prolonged than in case of a corresponding calcemia induced by other means; 3, the response is more pronounced in parathyroid deficient subjects than in normal subjects; 4, it is believed that the action of viosterol does not depend alone on stimulation of parathyroid tissue.

Rôle of the ovary in regulating the motility of the uterine fistula. SAMUEL R. M. REYNOLDS.

We have determined the activity of the uterine fistula 1, associated with oestrus and anoestrus in the unanesthetized rabbit; 2, in castrate female rabbits; and 3, in response to the injection of suitable oestrogenic preparations in the castrate rabbit.

a. *Uterine activity and oestrus:* Nineteen does have accepted the male or were known to be in heat. Eleven of these showed rhythmic, marked activity; six, moderate activity; and two, feeble activity. Of does not in heat, fourteen refused the male 23 times while showing feeble activity; seven refused the male 12 times while showing moderate activity. No does that showed marked activity refused the male.

b. *Uterine activity in the castrate:* Only does ovariectomized within a week of dropping a litter were used in castration experiments. Twenty of twenty-five does showed feeble irregular activity or were quiescent within a week of castration; five showed moderate, irregular activity. None showed marked activity. Ten of eleven does castrated for 21 days had uteri that were completely quiescent; one showed feeble irregular activity.

c. *Replacement therapy in castrates:* Four equal intravenous injections of Theelin were made in 8 hours. Two to 5 r.u. per kilo-body-weight in recent castrates restore activity temporarily within 15 to 24 hours. A

second response can be elicited when the activity has again subsided. Twenty or more r.u. per kilo of Theelin are necessary for a response in 21 day castrates. Fifteen r.u. are ineffective. The response to the lipid Estrogen duplicates that of the aqueous Theelin. Pituitary extracts and other tissue extracts used have never produced activity in quiescent uteri of castrate rabbits.

The results show 1, marked uterine activity is seen only in oestrus; 2, oestrus may occur infrequently when feeble or moderate activity is seen; 3, castration abolishes all activity; and 4, activity can be restored by appropriate substitution therapy with oestrogenic preparations. We conclude that the ovary imparts to the uterine fistula of the rabbit rhythmical contractions which are at times correlated with oestrus.

Studies in absorption from the gall bladder. III. Cholesterol and bile salts.

C. RIEGEL, C. G. JOHNSTON and I. S. RAVDIN.

A water suspension of cholesterol 0.1 to 0.3 per cent was introduced into the gall bladder. The results indicate that in this type of experiment little if any cholesterol is absorbed. When a solution of bile salt containing cholesterol in suspension was introduced into the gall bladder, cholesterol was absorbed.

Sodium taurocholate or sodium glycocholate in water or phosphate buffer was also used. The Gregory and Pascoe method for determining bile salts was used, the error being about 5 per cent. In all experiments the volume decreased and the total amount of bile salt decreased. However, the concentration of the solution increased since water was absorbed more rapidly than the bile salt.

Hemorrhage occurred in about 24 hours when bile salts were used alone. The combination of cholesterol and bile salts prevented this.

On the nervous mechanism of epinephrin secretion. J. M. ROGOFF, P. WASERMAN and R. HOECKER.

The relatively constant rate of epinephrin liberation from the adrenal glands, under ordinary experimental conditions, was found to be an average of about 0.00025 mgm. per kgm. of body weight per minute, in a large number of cats and dogs, when determined by measuring the epinephrin concentration in adrenal vein blood collected at a known rate of flow. This spontaneous (normal) liberation of epinephrin has been shown to be governed by a nervous mechanism situated in the upper dorsal region of the spinal cord. Hemisection of the cord in this region results in suppression of epinephrin discharge from the ipsilateral adrenal and transection affects both glands (Stewart and Rogoff, 1917, 1920).

When the functional integrity of the brain is interfered with, beyond the effects of ordinary anesthesia, (e.g., by compression, mechanical destruction or by anemia caused by ligation of the arteries to the brain) the rate of epinephrin secretion from the adrenals is within, but usually in the upper limit of, the normal range. This could be explained by the possibility of removal of an inhibitory influence of a mechanism in the brain, permitting the spinal cord mechanism to exercise its maximum influence. Experimental evidence has been obtained to support this view.

If the rate of epinephrin liberation is determined, in an animal under ordinary anesthesia and again after interference with the brain, the initial rate is usually found to be somewhere near the above mentioned average,

while the rate in the second instance is at or near the maximum of the "normal" range (Rogoff, 1924). Additional experiments have been performed. In these the animals (cats) were decerebrated at various levels. It was found that transection of the anterior part of the cerebrum does not materially alter the rate of epinephrin secretion, but decerebration in the region bounded by the superior colliculus and the optic chiasm results in the same degree of increase in the rate of epinephrin liberation from the adrenals as was observed in the other experiments already mentioned. If decerebration is followed by shock, the epinephrin output may fall to a level considerably lower than the initial rate.

These experiments may have a bearing upon the observation that, in certain experiments, strychnine may cause a *preliminary fall* followed by the usual large increase in the rate of epinephrin secretion (Stewart and Rogoff, 1919). It may also be suggested that if two opposing mechanisms are concerned in the liberation of epinephrin from the adrenals, this may explain the great difficulty in demonstrating reflex changes in the epinephrin secretion. This, and other, possibilities are reserved for further study.

The fetal influence on the basal metabolic rate. ALLAN WINTER ROWE and WILLIAM CLOUSER BOYD.

During the past ten years the senior author has been conducting serial studies on a large group of normal women during pregnancy. Weight and basal rate estimations at fortnightly intervals have defined a linear increase during the last twenty-four weeks at least. From the total heat produced, the maternal area and the fetal area as calculated from Scammon and Klein's formulae (Proc. Soc. Exp. Biol. and Med., 1930, xxvii, 461) it has been possible to estimate the fetal contribution. Other factors than increased maternal weight and fetal metabolism are apparently operative and these are discussed.

On the coördinating mechanism of the dog's uterus. LOUIS RUDOLPH and A. C. IVY.

We have described previously (1) a method for recording the contractions of the postpartum uterus of the dog *in situ*. While using this method we found that most of the movements started near the ovarian end of the horns and traveled downward to the corpus uteri or the fused portion of the dog's uterus. It occurred to us that by using this phenomenon of polarity of conduction, we might be able to throw some light on the nature of the coördinating mechanism of the uterus. (That such a mechanism does exist can hardly be denied on the basis of the observations on the uterus of the dog in labor¹.) We could ascertain, by separating the horns from the corpus, whether or not the polarity phenomenon persisted; and also by excising the utero-vaginal ganglion, we could ascertain if this structure was concerned.

A balloon was placed in either one or both horns and in the corpus uteri of dogs from eighteen to seventy-two hours postpartum. The record of normal contractions was obtained; then each horn was separated from the corpus and a record obtained; and then the utero-vaginal ganglia were excised prior to the separation of the horns. The necessary operative work can be done without disturbing the blood supply of the uterus. Experi-

¹ Rudolph and Ivy: Amer. Journ. Obstet. and Gynec., 1930, xix, 317.

ments have been performed so far on ten dogs. It is not difficult to find and excise the ganglia, because they are markedly hypertrophied by pregnancy.

We found that when both horns contracted simultaneously, a single contraction of the corpus would follow. When one horn contracted and was then followed shortly by the contraction of the other, a summation, or superposition effect was manifested by the corpus. When one horn contracted and was then followed a minute or so later by the contraction of the other, a double contraction of the corpus would result. In other words, the musculature of the corpus manifested the phenomenon of refractory period and summation, or superposition with reference to the propagated message of excitatory impulse.

Our observations also show that there is an intrinsic and an extrinsic (utero-vaginal ganglia) coordinating mechanism in the dog's uterus and that one may subserve, in part at least, the function of the other. An analogous type of coordinated polarity has been observed in the monkey by Ivy, Hartman and Koff.

Lack of isochronism between muscle and nerve. W. A. H. RUSHTON.

If a striated muscle is excited through large fluid electrodes, and the strength duration curve obtained, two excitable substances are found, one (γ) with chronaxie the same as that of nerve, and the other (α) with chronaxie very much greater. Now according to Lapicque's theory of isochronism, a single impulse should not be able to pass from the nerve to the α fibres, but that this does in fact occur may be seen with the aid of a slow heavy ballistic lever. Preliminary investigations showed that the excursion of the lever is proportional to the sum of the impulses upon it if they fall within 0.2 sec. Thus if the α substance can not respond to a single nerve impulse, the excursion resulting from a shock to the nerve followed at once by a direct stimulus to the α substance should be the same as the sum of the excursions due to these two excitations separately. The fact that it was considerably less shows that the α substance was rendered refractory by the nervous impulse. This was confirmed by separating the two stimuli by an interval of 60 σ which increased the excursion to the required sum, showing that at least some of the α fibres contract to a single nerve impulse. This makes it hard to believe that identity of chronaxie is a necessary condition for conduction between two tissues, for in the present case the two chronaxies may differ in the ratio of 100 : 1.

Epinephrine on muscle and liver glycogen. JACOB SACKS.

Using the method of Davenport and Davenport of freezing the muscles in situ with CO₂ snow and ether, glycogen was determined in the liver and muscles of cats previously starved twenty-four hours, 1½ and 3 hours after the subcutaneous injection of epinephrine. Anesthesia was produced by amytal or nembutal. In all, 25 animals were used.

A dose of 1.0 mgm. of epinephrine per kilo caused a marked decrease in liver glycogen and a slight decrease in muscle glycogen. A dose of 0.1 mgm. per kilo produced a slight decrease in liver glycogen and had no effect on the glycogen content of the muscles.

Under amytal anesthesia, one-fourth of the liver glycogen of control animals disappears in three hours.

The effect of carbon monoxide on the after-potential of medullated nerve.

FRANCIS O. SCHMITT and HERBERT S. GASSER.

It is known that the after-potential in green frog nerves is affected differentially from the spike potential when the nerve is placed in pure nitrogen (Amberson, 1930). It has also been shown that the oxygen needed for nerve respiration and for the conduction of the action potential has first to be activated by a catalyst similar to the respiratory enzyme of Warburg (Schmitt, 1930). The present investigation is designed to determine the rôle of this catalyst as regards the production of the after-potential.

Utilizing the light-sensitivity of the carbon monoxide-poisoned catalyst, it has been demonstrated that, as in the case of the spike of the action potential, the after potential requires oxygen activated by this catalyst. When a nerve is placed in the carbon monoxide-oxygen mixture in the dark, the after-potential decreases much more rapidly than the spike and appears to go to extinction at a time when the latter has decreased to but 30-50 per cent of its original value. Illumination of the nerve at this juncture causes a prompt increase both of the after-potential and of the spike, the former, however, rising to its maximum considerably before the latter. When illumination is discontinued the after-potential decreases very rapidly and disappears in but a few minutes while the spike potential decreases slowly and does not disappear for perhaps an hour or so.

Poisoning a nerve with veratrin is known to increase greatly the after-potential. When a nerve so poisoned is treated in the manner described above, the same cycle of events may be demonstrated still more strikingly.

A study of responses to work on a bicycle ergometer. EDWARD C. SCHNEIDER.

Six men of sedentary habits carried loads of 2000, 4000, 6000, 8000 and 10,000 foot-pounds. A linear relationship between the amount of oxygen absorbed and the load of work was maintained by all during moderate exertion but in four cases this relationship was broken soon after the crest-load was passed. The pulse frequency likewise augmented roughly proportional to the increase in work up to a certain load which varied from man to man. Beyond this it accelerated in lesser degree. The oxygen-pulse rose steadily with increasing loads of work. Good reactors increased the oxygen-pulse beyond expectation when carrying the heaviest loads, while poor reactors failed entirely or made but little addition to it as their limit of load carrying was approached.

The minute-volume of breathing of four subjects maintained a linear relationship to the load of work up to the crest-load. Hyperventilation appeared above this. The frequency of breathing increased proportionately with the load up to or just above the crest-load, while with over-loads of work it augmented inordinately.

Evidence of failure in compensation occurred with heavy loads of work. In two instances the heart seemed to be incapable of meeting the demands. In two cases the unloading of oxygen from the blood did not augment as it did in the two men who made the best compensations. In only one case was there any likelihood that the respiration failed to respond to the demand made upon it.

Changes in temperature in the extremities of animals in relation to anesthesia and lumbar sympathetic ganglionectomy. CHARLES SHEARD, E. H. RYNEARSON and W. McK. CRAIG.

When the environmental temperature of the apparatus housing the

hind extremities is rapidly lowered from room temperature to the neighborhood of the freezing point, the records of temperatures obtained with thermocouples placed superficially between the toes of the hind feet of normal dogs indicate a marked drop in temperature which is maintained over a considerable period of time. Subcutaneous temperatures of the limbs, however, closely follow the changes in environmental temperature. Under ether anesthesia, both the subcutaneous and superficial temperature readings follow the same course and parallel, or exceed in rate, the changes in environmental temperature. In animals in which lumbar sympathetic ganglionectomy and trunk resection have been performed, no characteristic differences in the temperature-time relations are found, either subcutaneously or superficially, when the animal is under ether. Without ether anesthesia the post-operative subcutaneous temperature-time relations are analogous in all respects to the pre-operative readings. The temperature-time relations of the superficial temperatures of the foot on the unoperated side are the same as before operation and indicate the presence of vasoconstriction when the environmental temperature is lowered. Similar readings on the foot of the operated side show the usual decrease of temperature followed by a relatively rapid return to normal and indicate the absence of vasoconstriction.

The lactic acid cycle in excised mammalian muscle. EPHRAIM SHORR, ROBERT O. LOEBEL and HENRY B. RICHARDSON.

The cycle $\text{glycogen} \rightleftharpoons \text{lactic acid}$, established as so important for frog muscle, has never been definitely demonstrated in mammalian muscle. These studies were made in order to ascertain whether this cycle existed in the voluntary muscle of the normal dog.

Methods. Muscle strips of the proper thickness were prepared by the technic previously described by us, from neck muscles removed under ether anesthesia. Preliminary to the study of their gaseous metabolism in the Barcroft-Warburg microrespiration apparatus, these strips were washed in physiologic saline through which oxygen was bubbled, to reduce the content of preformed lactic acid. Oxygen consumption and respiratory quotients were then determined simultaneously, a , in a non-nutrient physiologic saline solution buffered to pH 7.4 by phosphate, and b , in the presence of racemic sodium lactate. The changes in the lactic acid content of the tissues under these conditions were followed by chemical analysis. Four oxidative quotients were also obtained from the simultaneous study of the respiratory and lactic acid metabolism in oxygen, and the lactic acid production in nitrogen. They were done in 0.9 per cent saline solutions buffered by phosphate, which contained 0.2 per cent glucose in two of the experiments.

Results: Stimulation of respiration by lactate. With this technic, sodium lactate caused a stimulation of respiration in every experiment, which averaged 44 per cent for the series.

Effect of sodium lactate on the respiratory quotient. The average of the non-nutrient respiratory quotients was 0.94, of those in lactate 0.91. Thus there was a slight decline (-0.03) in the respiratory quotient in the presence of lactate instead of the rise ($+0.03$) which would have followed if the oxygen consumption in lactate were at the expense of lactate. This may mean that the stimulation of oxidation by lactate was non-specific. However, the theoretical changes were small enough to have been masked by experimental errors.

The oxidative quotient. It is customary to derive the oxidative quotient from two equations:

(1)

$$\text{Ox} \cdot \text{Q.} = \frac{\text{HL disappearance in lactate} - \text{HL disappearance in non-nutrient medium}}{\text{HL equivalent of extra oxygen consumption in lactate}}$$

(2)

$$\text{Ox} \cdot \text{Q.} = \frac{\text{HL appearing in Nitrogen} + \text{HL which would have disappeared in Oxygen}}{\text{HL equivalent of total oxygen consumption}}$$

TABLE 1

Oxidative quotient of washed muscle strips of the normal dog. Derived from aerobic disappearance of lactic acid. Effect of lactate on R.Q. and respiration

Two hour periods

EXPERIMENT NUMBER	NaL conc. mgm./100 cc.	OXYGEN CONSUMPTION			RESPIRATORY QUOTIENT			OXIDATIVE QUOTIENT
		Non-nutrient	With lactate	Change	Non-nutrient	With lactate	Change	
		cc. per moist gm.	cc. per moist gm.	per cent				
1	165	0.45	0.57	+27				7.2
2	90	0.42	0.55	+31				
3	145	0.48	0.65	+36	0.85	0.85	0.00	6.8
4	170	0.43	0.60	+40		0.88*		4.1
5	155	0.46	0.66	+44	1.01	0.96	-0.05	3.5
6	170	0.38	0.57	+50	0.96	0.92	-0.04	2.0
7	135	0.57	0.99	+74	1.00*			2.4
Average....	149 (app. m/80)	0.46	0.66	+44	0.94	0.91	-0.03	4.3

* Excluded from averages.

TABLE 2

Oxidative quotient of washed muscle strips of the normal dog. Derived from anaerobic production of lactic acid in saline-phosphate solution

Two hour periods

EXPERIMENT NUMBER	CONTROL TISSUE IN OXYGEN			CHANGE IN TISSUE LACTIC ACID		OXIDATIVE QUOTIENT
	R.Q.	Oxygen consumption	HL equivalent of total oxygen consumption	In oxygen	In nitrogen	
		mm. per moist gm.	mgm. per moist gm.	mgm. per gram	mgm. per gram	
5	1.01	380	0.51	-0.41	+0.89	2.6
8*	0.95	410	0.55	+0.03	+1.74	3.1
9*	0.94	780	1.04	-0.40	+3.40	3.7
10†	0.98	290	0.39	-0.18	+1.33	3.9
Average....	0.97					3.3

* Received 0.2 per cent glucose. Experiments 5 and 10 done in saline-phosphate without the addition of glucose.

† 1 hour run.

Quotients above 1.0 indicate in each instance that more lactic acid disappears than can be accounted for by oxidation.

In these experiments the first type of oxidative quotient varied from 2.0 to 7.2, and averaged 4.3 (table 1). The second type of oxidative quotient varied from 2.6 to 3.9 and averaged 3.3 (table 2). Oxidation could therefore account for only about $\frac{1}{4}$ of the lactate which disappeared. The ultimate fate of the remaining $\frac{3}{4}$, presumably its conversion into the carbohydrate stores of the muscle, was not determined in this series.

The response of the kidney to strict limitation of mineral salts in the ration.

ARTHUR H. SMITH and PEARL P. SWANSON.

The strict limitation of inorganic salts in an otherwise adequate experimental diet is accompanied by significant changes in the fresh weight and water content of the kidneys. The body weight of the experimental rats is maintained at a fairly uniform level. After 21 days on the salt-poor diet the kidneys show a marked increase in size over those of the normal controls. The relative content of water of the kidneys at this time is not abnormal. After 42 days the kidneys of the experimental rats have not changed from the size at 21 days while those of the normal controls show the usual increase with growth. However, the weight of the "low-salt" kidney is maintained at this level by water, since the percentage solids has decreased. At 63 days and at 90 days the fresh weight decreases progressively while the kidneys of the normal controls continue to increase. The diminution of fresh weight of the "low-salt" kidneys at these periods is due to a parallel decrease of both solids and water though there is significantly more water than in the normal kidney. After 90 days on the salt-poor ration the fresh weight of the kidneys is less than that of kidneys of normal animals of the same age but more than that of normal rats of the same body weights.

Studies on the mechanism of the pain in peptic ulcer. FRED M. SMITH and W. D. PAUL.

The method employed was similar to that reported in the investigation of the mechanism of the gastric pain associated with irritable colon and chronic appendicitis. The activity of the pyloric section of the stomach was recorded by means of a rubber balloon connected through a water system with a kymograph.

In every instance studied, the pain corresponded with an increase in tone and the passage of a peristaltic wave over the pyloric section of the stomach. The most severe pain was associated with violent tone changes. Relief from the distress was accompanied by a reduction in the tone and a diminution or cessation of the peristaltic waves. In those in whom relief was obtained by the administration of atropin, alkalies, and other agents, similar changes in the gastric activity were noted. If, on the other hand, the distress was intensified, there was always an associated increase in the tone and peristalsis.

These results indicate that the pain in peptic ulcer is induced by an increase in the tension of the pyloric section of the stomach and the mechanism is similar to that of the gastric pain associated with irritable colon and chronic appendicitis.

The temperature coefficients of the several processes in muscle contraction.

PAUL W. SMITH and MAURICE B. VISSCHER.

Muscle contraction involves a series of reactions, each of which may be influenced differently by temperature. It has been possible to unravel the process and pick out the temperature coefficients of several components.

By measuring lactic acid liberated in repeated single twitches it is observed that when there is a negative temperature coefficient for tension set up, there is also a negative temperature coefficient for lactic acid production, as the accompanying examples show.

CONTRACTIONS AT 10°C.		CONTRACTIONS AT 30°C.		$\frac{\Delta \text{L. A.}}{\Delta T}$	$\frac{\Delta \text{Tension}}{\Delta T}$
Tension per contraction	Lactic acid per contraction	Tension per contraction	Lactic acid per contraction		
grams	mgm. per cent	grams	mgm. per cent		
800	1.26	480	0.80	-0.023	-16
910	1.33	510	0.85	-0.024	-20
730	1.25	520	0.97	-0.014	-11
620	1.37	350	0.85	-0.026	-14

When one computes the coefficient relating tension set up to lactic acid liberated, which is a measure of the economy of setting up tension, one finds, however, that its differential with respect to temperature is zero. That is, the tension set up in contraction per unit of lactic acid formed is independent of the temperature.

On the other hand, acid contracture has a positive temperature coefficient, indicating that if lactic acid is the causative agent in contraction there should be a positive temperature coefficient for tension produced per unit of lactic acid, other factors being ruled out. We have investigated one of these other factors, namely, the rate of anaerobic recovery from contraction, and find that it is proceeding during contraction itself, and has a very high positive temperature coefficient, $Q_{10}=3$. Therefore the tension-producing agent seems to be disappearing even while it is producing tension and consequently the whole amount liberated in a contraction would not be present at the time of maximum tension. It is to be inferred, therefore, that the ultimate absence of effect of temperature on the isometric tension coefficient of lactic acid in twitches is due to the fact that the two separate effects balance each other.

It is apparent that the ultimate effect of temperature upon contraction is a composite of the effects upon the separate processes.

Autoxidation of fats. E. I. SOLLMANN.

The close similarity between oxidation of fat in the animal organism and of naturally occurring fats and oils by oxygen prompted a more detailed study of the latter.

The oxygen absorption method has been used almost exclusively in this field, but the information gained has been lacking in details. The observation that there are present in oxidized fats substances capable of liberating iodine from acidified alcoholic potassium iodide led to the development of a method utilizing this property to determine the amount of oxidation that has occurred. The liberated iodine is titrated with 0.01 N sodium thio-sulphate. Cobaltic oleate and peracetic acid have been used as catalysts, the oil being mixed with the catalyst and exposed to oxygen.

The following oils were studied: sesame, palm, soya, cotton-seed, oleo, and corn oil. The data obtained indicate that the oxidation of fats and oils in vitro is autocatalytic in character, this autocatalysis being effected by light, heat and free organic acids. Since it appears quite likely that the "mol-oxides" are not the active agents in question, a tentative theory is proposed, involving the catalytic activity of complex metallic acids and organic per-acids and per-esters.

Because cottonseed oil showed the greatest susceptibility to oxidation, it was used in further studies on anti-oxidants. (The resistance to oxidation shown by the corn and soya oils used was found to be due to small amounts of sitosterol and lecithin.) Using cottonseed oil and cobaltic oleate as catalyst, a number of substances were tested for anticatalytic action: cholesterol, stigmasterol, sitosterol, lecithin, diphenyl guanidine, potassium cyanide, hydroquinone, and ammonia. Lecithin was the most powerful anti-oxidant. Of the sterols used, only sitosterol exhibited any antioxidant properties. In general, the autocatalysis is inhibited by substances of a basic nature, those possessing *active* hydroxyl or amino groups.

Results with the oxygen absorption method, using the Warburg apparatus, indicate also that lecithin inhibits the autoxidation of cotton-seed oil. Further, lecithin subjected to oxygen and temperatures above 65°C. for one-half hour no longer exhibits any inhibitory action as an antioxidant.

A new method for perfusing the isolated mammalian heart. SAMUEL SOSKIN and LOUIS N. KATZ.

This apparatus and method are designed to measure the useful external work performed by both ventricles of the heart, individually or together. The work performed is determined from the amount of blood pumped by the heart to a set height. The oxygen consumption and the work efficiency can be computed from gaseous analyses of inflowing and outflowing blood. The filling pressure of the coronary vessels and the two auricles, and the resistance to emptying can be controlled independently of each other and kept constant. The inflowing blood is conducted through cannulae into the superior vena cava and the left auricle, and into the coronaries through the aorta. The blood leaves the heart through cannulae inserted through the ventricular walls. On the tubes leading from these cannulae, artificial valves replace the semilunars. This preparation is being used to test the action of hormones and drugs upon the heart muscle.

The prevention of hypertrophy and the limitation of normal pulsation of the kidney by means of casts. SAMUEL SOSKIN and OTTO SAPHIR.

In a series of dogs, one or both kidneys were enclosed in rigid casts, applied in the form of narrow gauze bandage soaked in collodion and allowed to harden in situ. The main purpose in view was to observe the effect of such a cast on a kidney stimulated to hypertrophy by the simultaneous or subsequent removal of the opposite kidney. Such animals invariably died, presumably from kidney insufficiency. Histological examination of such kidneys showed only a moderate cloudy swelling or fatty degeneration, not commensurate with the failure of function. Control experiments indicated that the results were not due to the trauma of the operation or the irritant effect of foreign material about the kidney. Thus, when casts applied some weeks previously were split or removed, the animals survived the removal of the opposite kidney at a subsequent operation.

No severe or permanent damage could, therefore, have been caused by the cast.

It was observed, however, that the application of a cast to one kidney was followed by hypertrophy of the opposite kidney, indicating an impairment in function while the cast was in place. But, as pointed out above, there was no extensive morphologic damage, and such a kidney, once the cast was removed, could alone carry on sufficient function to maintain the life of the animal. These results seem to indicate that a normal physiologic condition necessary to kidney function was interfered with. It is suggested that this factor may be the normal pulsation of the kidney coincident with the pulse pressure, or the increase in volume due to increased blood flow accompanying active secretion.

Action of quinine and amytal on the salivary secretion. GEORGE STAVRAKY.

Intravenous injections of quinine hydrochloride (0.25 to 0.5 gm.) in a dog diminish the secretion activated by the chorda tympani and in larger doses arrest it entirely.

Quinine inhibits the secretory effect of choline and acetyl-choline on the submaxillary gland, but does not interfere with the action of pilocarpine. It also inhibits the vasoconstrictor effect of the sympathetic nerve on this gland, but increases its secretory effect.

Intravenous injection of physostigmine hydrochloride (in subminimal doses) restores the secretory effect of the chorda tympani.

Intra-abdominal and intravenous injections of amytal decrease the secretory effect of the chorda tympani and of choline and acetyl-choline on the submaxillary gland. The action of pilocarpine is not affected by this drug. Amytal also diminishes the secretory and vaso-constrictor effect of the sympathetic nerve on the submaxillary gland, although in a lesser degree than in the case of the parasympathetic nerve.

Studies in the physiology of exercise. VI. The influence of treadmill running on the motility of the dog's colon. ARTHUR H. STEINHAUS and VERNON R. DE YOUNG.

In dogs completely recovered from operations to produce various suitable fistuli of the colon, motility was determined by the balloon method. From a comparison of tracings secured while the dog stood at rest on the treadmill with those secured during brief periods of running and subsequent resting periods the following generalizations emerge.

1. A brief period (usually six minutes) of strenuous running almost invariably brings on a very marked increase in colonic motility. This period of augmented activity is *not* coextensive with the running period. It comes on after a definite latent period and recedes usually before exercise is discontinued.

2. Following the exercise period there is usually a period of subnormal motility and tone which persists for a varying length of time—sometimes as long as twenty minutes.

3. From a series in which various denervation techniques were employed we conclude that the augmented motility during exercise is dependent on the intactness of certain extrinsic nerves.

4. In a badly frightened dog who "fought" the treadmill the augmentation did not occur until after he had learned to run without this emotional reaction.

Studies on the physiology of secretin. IV. The effect on the secretion of bile.

EUGENE U. STILL, J. B. McBEAN and F. A. RIES.

We have endeavored to determine if the flow of bile caused by the intravenous injection of secretin preparations is caused by secretin, *per se*, or some contaminating substance.

We have found the following: 1. The injection of such quantities of various secretin preparations (crude and purified) as will cause similar pancreatic responses causes similar increases in the flow of bile. 2. The treatment of secretin with reagents which nullify the pancreatic activity also nullifies the liver activity. 3. The rate of destruction of the two activities by reagents is similar. 4. On injection of secretin or the application of HCl to the duodenum (the quantities being adjusted so as to obtain similar pancreatic responses) the quantities of bile secreted in the two cases are similar. 5. The intravenous injection of secretin will increase the pressure against which the liver can secrete. 6. The chologogue action of secretin is effective in eviscerated animals. 7. The injection of massive doses (100-200 secretagogue doses) does not alter the vasomotor state of the liver as measured by the plethysmograph. 8. A small dose (1-5 mgm.) of purified secretin is a more potent chologogue than a similar dose of bile salts. Increasing the dosage of secretin does not materially increase the liver response while larger doses of bile salts increase the liver response roughly in proportion to the size of the dose.

We believe our observations warrant the conclusion that secretin *per se* is a chologogue which is active by virtue of the stimulation of the hepatic cells.

The effects of low environmental temperature upon metabolism. R. W. SWIFT.

Following the determination of basal metabolism by the Tissot method, human subjects, lightly clad, were exposed to a temperature of about 1°C. for 75 minutes. Three samples of expired air were obtained during each experiment representing the periods of 20, 45, and 70 minutes' exposure to the cold.

Duplicate experiments on the same individual on different days showed good agreement but the response of one individual as compared with that of another reveals prominent differences. These differences are associated with the amount of subcutaneous fat present, measurements of which have been taken. A thick layer of subcutaneous fat delays the onset of shivering and no marked increase of heat production takes place before shivering begins.

The respiratory quotient usually rose slightly during the first 20 minutes and then slightly decreased. No significant changes have thus far been found in the nitrogen elimination nor in the blood sugar. The heart rate was constant in a few cases and decreased in most. The respiratory rate decreased slightly while the respiratory volume increased markedly showing an excellent correlation between the volume of expired air and heat production. It seems probable that no increase in heat production takes place without increase in muscular tonus which precedes shivering. *Rectal temperatures indicated a drop in body temperature in most cases amounting to 0.1 to 0.2°C. The stimulus for shivering did not consist of even a slight drop in body (rectal) temperature. Shivering began when the skin temperature had dropped to about 19°C.

The cortical hormone of the adrenal gland. W. W. SWINGLE and J. J. PFIFFNER.

The adrenal cortex of mammals contains a hormone necessary for the maintenance of life. This hormone can be extracted from adrenal glands by suitable agents and obtained in relatively pure form, free from proteins, lipids and other contaminating substances. The extract is suitable for intravenous use and is non-toxic in large doses.

1. Administration of the hormone to bilaterally adrenalectomized cats prolongs the life span indefinitely. The animals remain in normal condition, gain weight, exhibit normal sex behavior, and cannot be distinguished from control unoperated cats. Such treated animals succumb with typical symptoms of adrenal insufficiency following withdrawal of the extract treatment.

2. Prostrate cats, on the verge of death from adrenal insufficiency, can be restored to normal within 48 to 72 hours by injecting small amounts of the extract. After return to normal condition the cats can be kept so for any length of time desired, by a daily subcutaneous injection of small amounts of the extract.

3. The extract has been used successfully in the crises of Addison's disease. Intravenous injections revive prostrate individuals and restore them to apparent normal activity.

4. Data pertaining to the function and chemical properties of the cortical hormone were presented.

The source of the excess calcium in the sera of dogs receiving large doses of irradiated ergosterol. N. B. TAYLOR and C. B. WELD.

There are several points of resemblance between the action of parathormone and that of large doses of irradiated ergosterol. Taylor, Branion and Kay,¹ for instance, reported that the post-mortem findings in the gastrointestinal tract so characteristic of parathyroid over-dosage in dogs were identical with those resulting from the administration of irradiated ergosterol. The rise in serum calcium, the decalcification of the bones and the increased excretion of calcium in the urine are also features common to the action of both these materials. It seems to be definitely established that the rise in the calcium of the serum following parathormone is at the expense of the bone calcium. The source of the excess calcium in the serum resulting from ergosterol overdosage has, however, been a subject of dispute. See Hess, Weinstock and Rivkin;² Jones, Rapoport and Hodes.³

With a view of securing evidence which might enable one to decide whether the calcium stores of the body or of the food supplied the excess calcium, calcium balances were carried out upon dogs fed calcium deficient diets. A pronounced negative balance was induced by means of thyroid extract. During a period when large quantities of calcium were being lost in the feces, maximal daily doses of irradiated ergosterol (1 cc. 10.000 X) were given by mouth. Nevertheless, the serum calcium rose to 75 or 100 per cent above normal. In subsequent experiments it was found unnecessary to give thyroid extract, since the sterol itself caused a pronounced negative calcium balance, accompanied by hypercalcemia. In one such experiment, extending over a period of 6 days, the calcium balance was

¹ Taylor, Branion and Kay: *Journ. Physiol.*, 1930, lxi, Proc.

² Hess, Weinstock and Rivkin: *Proc. Soc. Exp. Biol. and Med.*, 1929, xxvi, 199.

³ Jones, Rapoport and Hodes: *Journ. Biol. Chem.*, 1930, lxxxix, 647.

-653.8, yet the serum contained some 16 mgm. of excess calcium. It was also found that the intravenous injection of irradiated ergosterol into eviscerated cats caused a rise of from 40 per cent to 100 per cent in the serum calcium within 3 or 4 hours.

Effect of viosterol on excretion of nitrogen, calcium and phosphorus in normal dogs. E. A. THACKER and L. M. DILLMAN.

Intravenous injections of viosterol in doses ranging from 0.75 to 12 cc., of preparation of 100 D potency per kilo of body weight were made in dogs trained to the metabolism cage and fed on a standard balanced ration, analysed for N, Ca and P content. With the larger doses no constant results could be obtained because of the induction of toxic symptoms. With optimal doses, total N excretion was usually increased for a short time but a positive balance was maintained. Later there was a decrease sometimes below the original level. Total P excretion also was increased briefly but declined slightly. Total Ca excretion was decreased slightly with a positive balance. After discontinuing administration, there was a brief increased excretion with negative balance.

A simple method for controlling the pH of oxygenated Locke's solutions. J. EARL THOMAS.

The ideal method of preventing the loss of carbon dioxide and consequent change of pH that results from ventilating bicarbonate buffered solutions, such as Locke's solution, with air or oxygen, is to add carbon dioxide in appropriate concentration to the ventilating gas supply. The following observation makes available a method for accomplishing this without elaborate apparatus or exacting quantitative determinations.

Powdered magnesium carbonate, suspended in solutions of the alkali phosphates, decomposes so as to maintain a constant carbon dioxide tension in the mixture over a long period of time and in spite of the loss of considerable carbon dioxide. The carbon dioxide tension of the mixture depends upon the total concentration of phosphate and is relatively independent of the initial pH of the mixture. Air or oxygen that is in equilibrium with such a mixture will, when bubbled through a Locke's solution, establish and maintain a constant carbon dioxide tension, hence a constant pH, in the Locke's solution. Mixtures can be prepared which, when used in this way, will serve to maintain any desired pH between 6.0 and 8.3 in a standard Locke's solution, containing 0.015 per cent sodium bicarbonate.

In practice, sodium and potassium phosphate solutions are prepared in the concentrations and mixed in the approximate proportions indicated in the table as providing for the desired pH in the Locke's solution. About 25 grams of powdered magnesium carbonate are added to each 450 ml. of phosphate mixture and the phosphate-carbonate mixture allowed to stand in an open flask for several hours, preferably over night. Two or more gas washing bottles are partly filled with the mixture and connected into the air or oxygen line through which the Locke's solution is being ventilated. The mixtures that serve to maintain a Locke's solution at pH 7.4 or higher can be used for several days without noticeable change.

The following table gives the information necessary for the preparation of the phosphate mixtures. The data are for room temperatures.

MOLAR CONCENTRATION OF PHOSPHATE	AMOUNT OF EACH SOLUTION			pH TO BE MAINTAINED IN LOCKE'S (0.015 PER CENT NaHCO ₃) (add 0.2 for 38°C.)
	K ₂ HPO ₄	Na ₂ HPO ₄	NaH ₂ PO ₄	
	ml.	ml.	ml.	
2.0	375		75	5.9
1.75	383		67	6.1
1.5	400		50	6.2
1.25	410		40	6.45
1.0	420		30	6.8
0.75	430		20	7.0
0.5	430		20	7.3
0.25	435		15	7.5
0.5		400	50	7.4
0.4		400	50	7.6
0.3		410	40	7.8
0.25		420	30	7.85
0.2		420	30	8.0
0.1		430	20	8.3

The effect of the vagus on the diastolic size of the heart and its bearing on the question of cardiac tonus. EDWARD J. VAN LIERE and GEORGE CRISLER.

A series of eight barbitalized dogs was used. The trachea was cannulated and the right vagus exposed and cut. An x-ray picture of the heart was obtained using a constant target film distance of one meter. A second x-ray picture was made during vagus stimulation to establish the functional reliability of this nerve. The intrapulmonic pressure was raised to 120 mm. or more of water by blowing into the trachea and clamping it. A control series of pictures was obtained after 10, 30 and 50 seconds. The tracheal cannula was unclamped and the animal allowed several minutes to recover. A second series of x-ray pictures was then taken at the same time intervals but the vagus was stimulated at the beginning of the 30 second period.

The area of the cardiac silhouettes was measured in each case. It was found that the size of the heart when the vagus was stimulated in the experimental series was no greater than in the control series.

The increased intrapulmonic pressure prevented blood from entering the heart and so the venous pressure was controlled. This is necessary as it is well recognized that when the vagus is stimulated the heart is slowed or stopped which allows the venous pressure to balloon out the heart. The fact that the heart did not change in size when the vagus was stimulated and the venous pressure was controlled as above described allows the conclusion to be drawn that whether or not there is such a thing as cardiac tonus in the mammalian heart, the vagus nerve seemingly does not function in the mechanism.

Experiments on acid-base equilibrium in relation to gastric secretion. ARTHUR M. VINEBERG and J. S. L. BROWNE.

A flow of gastric juice was obtained by vagal stimulation in dogs. At its maximum artificial ventilation was applied, with consequent reduction of total acid output. This output was restored to previous values by the use of CO₂-oxygen mixture as the inspired air.

The blood CO₂ content, pH, chlorides, lactic acid, and total bases were estimated on arterial blood. The volume, free and total acid and total chloride were estimated on gastric secretion.

The rate of gastric secretion can be correlated with the level of CO_2 in the blood.

The presence of a soluble mucoprotein in the gastric juice. D. R. WEBSTER and S. A. KOMAROV.

It was observed that varying amounts of organic material were present in the pure gastric juice under different conditions of stimulation. Analysis indicated one of the constituents of this organic material to be a soluble mucoprotein. Outline of the analytical data and the quantitative estimation under different conditions.

The value of oysters in nutritional anemia. DOROTHY V. WHIPPLE and OPAL M. WOLF.

The hemoglobin building power of dried beef liver was compared with that of dried white oyster and dried "green oyster."

Rats suffering from a nutritional anemia in which the average hemoglobin is below 4 grams per 100 cc. of blood respond rapidly to daily doses of 0.5 gram of dried beef liver and dried white and green oyster. At the end of 16 weeks on the supplements the average hemoglobins rose to the following figures:

Liver.....	13.46
White oyster.....	11.47
Green oyster.....	13.40

The control animals all died before 50 days with hemoglobin values too low to read on the colorimeter, namely, below 2.0 grams. These figures represent data from 54 animals, 25 controls on milk alone, 13 milk and green oyster, 8 milk and white oyster and 8 milk and liver.

In a similar experiment using 1.0 gram instead of 0.5 gram the animals respond more rapidly reaching the high point at 28 days. Factors of heredity, age, and prenatal store of metals were controlled by using litter mate controls on all experiments.

A nutritional anemia was produced by feeding to adult females a diet of two-thirds whole wheat and one-third powdered milk supplemented with a small piece of fresh beef muscle and lettuce daily. The young from these females were placed at weaning on raw whole fresh milk ad lib. The hemoglobin of the young went down gradually and when the average value was below 4 grams per 100 cc. of blood they were started on supplemental feedings as detailed above.

The type of green oysters used in this experiment are found in certain locations along the Atlantic Coast. They are characterized by a greenish color especially marked along the blood vessels and have a higher mineral content than the ordinary white oyster.

Characteristic differences in the aortic and ventricular pulse curves with aortic leaks of different sizes. CARL J. WIGGERS.

The changes in aortic pressure pulses after experimental production of aortic leaks of different sizes were reinvestigated with a view of discovering essential differences of possible practical significance. They were also compared with intraventricular pressure curves with the two-fold hopes of further elucidating the mechanisms of regurgitation and the share that the physiological compensation plays in the dynamic changes in the circulation.

Optical records of aortic pressures following leaks of different sizes present many similarities; the amplitude is markedly increased, the ascent is steeper, the systolic pressure often exceeds the normal and irregular vibrations are frequently superimposed on the basic pressure patterns. In addition, the decline of pressure during the last portion of ejection is steeper and greater. These effects are partly accounted for by the low arterial resistance at the onset of ejection, but they are accentuated or modified by the increased force of contraction and augmented systolic discharge resulting from a rise in initial tension.

But leaks of different sizes cause many dissimilarities as well. Thus with leaks of moderate size, the isometric phase *shortens* and ejection phase *lengthens*, but different portions of the ventricular pressure curves retain their normal slopes and characteristics. The chief decline of aortic pressure occurs *after* the incisura, and aortic pressure continues to decline after natural ventricular filling has begun. On the other hand with extremely large leaks, the phase of isometric contraction barely exists. The ascending and descending slopes of the ventricular pressure curve are less perpendicular and lose their characteristic changes in gradient. The chief fall of aortic pressure occurs *during* the incisura which imperceptibly continues into the isometric relaxation phase; in other words the pressure practically reaches its lowest level by the end of isometric relaxation and previous to natural ventricular filling from the left auricle. These differences are due to the fact that even partial closure of the aortic valves offers considerable resistance both to ejection and backflow. Hence, the left ventricle contracts as an afterloaded muscle unless leaks of extreme degree exist; in which case it of course contracts as a loaded muscle.

The failure of a super-maintenance diet to increase the total transformation of energy per square meter of body surface. F. H. WILEY.

An unusually thin, otherwise normal, young man was fed a diet intended to be without effect on his body weight. For a period of 18 days the total available energy of the diet was found to be equal to the total transformation of energy. The energy of the diet was then increased about 60 per cent, and the transformation of energy was then determined over a period of 15 days. There was no increase in energy transformation per square meter of body surface. The basal metabolic rate in the first period was -10 per cent. It quickly rose to normal in the second period and then remained at that value. The gain in weight during the second period is evidence of the storage of the extra energy of the diet.

The initial effect of vasoconstriction on the concentration of the blood. L. B. WINKELSTEIN and F. H. SCOTT.

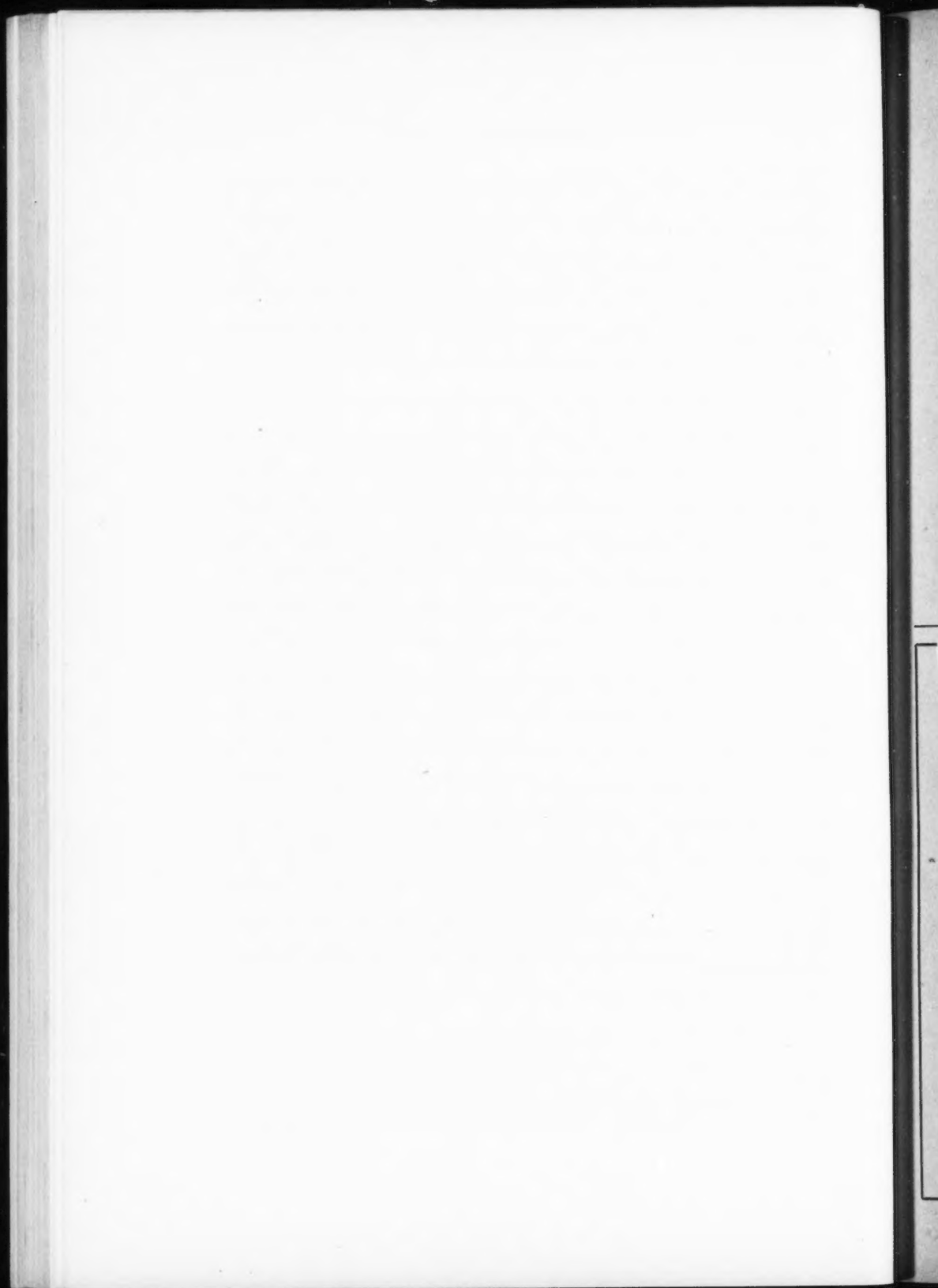
A number of conditions such as asphyxia or epinephrin are known ultimately to produce a great concentration of the blood, either by the loss of fluid from the blood or by the addition of new corpuscles from organs, especially from the spleen. Most of the conditions known to produce these concentrations of blood are accompanied by a great vasoconstriction. The measurements of capillary pressure in recent years by Landis have made it probable that fluid is filtered out in the arterial end of the capillary into the tissue spaces while at the venous end of the capillary there is a return of fluid to the capillary due to higher osmotic pressure of the colloids of the blood. (Starling's theory of lymph production.) Like-

wise, the measurements show that a vasoconstriction reduces capillary pressures. If this be true, the initial effect of a vasoconstriction over a large area should be a dilution of the blood. This we have found to be true with such things as adrenalin, asphyxia and hemorrhage. Thus if one considers 100 as the concentration of the hemoglobin in the blood before the injection of adrenalin, about one minute after the blood pressure commences to rise it drops to about 95. The same is true for asphyxia and has been found in a few cases of hemorrhage. The further effect on the blood and the differences between different procedures depends on the different effects they produce in different organs. The presence or absence of the liver or spleen makes little difference in this initial effect.

Vascular responses to histamine in suprarenalectomized rats. LELAND C. WYMAN and CAROLINE TUM SUDEN.

Intravenous injections of histamine produced depressor responses to all doses tried, the minimal effective dose being about 0.0001 mgm. Microscopic observation of intestinal blood vessels showed only dilator reactions to intravenous histamine. Increased susceptibility to histamine poisoning follows immediately the removal of the influence of the suprarenal medulla from the circulation by excision or ligation of the glands. The minimal intravenous dose of histamine necessary to produce a permanent fall of blood pressure is from 0.3 to 0.5 mgm. per 100 grams of body weight for normal or blank operated rats, and from 0.05 to 0.1 mgm. per 100 grams for suprarenalectomized rats, or rats having autoplasmic cortical transplants or gross accessory cortical tissue but no demonstrable chromaffin tissue.

Subcutaneous injections of adrenalin, 0.02 mgm. per 100 grams body weight, twice daily during seven days following suprarenalectomy, the last injection being given two hours prior to the injection of histamine, did not materially affect the vascular responses. The majority of these rats showed responses similar to those of untreated rats having no demonstrable chromaffin tissue. In a few cases the initial blood pressure was higher and the reactions to histamine not so marked as in the case of suprarenalectomized rats with no previous treatment. Exactly similar results were obtained, however, in a series of suprarenalectomized rats which received a single subcutaneous injection of adrenalin two hours prior to the injection of histamine, indicating that in the first series the last injection of adrenalin may have exerted a pharmacological effect in some cases on the subsequent responses to histamine. It is believed, therefore, that the influence of the suprarenal medulla in protecting the rat from the fatal effects of histamine poisoning is effective by virtue of the antagonism between secreted adrenalin and histamine, rather than that the protective action of adrenalin is dependent on a continuous effect maintaining the nutrition or tone of some part of the vascularneuromuscular mechanism.



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